# Studies on the control of growth and development in Macadamia integrifolia (Maiden and Betche)

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# STUDIES ON THE CONTROL OF GROWTH AND DEVELOPMENT IN MACADAMIA INTEGRIFOLIA (Maiden and Betche).

Submitted by Ibrahim Makoka Green Phiri for the degree of Ph.D of the University of Bath 1991.



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## SUMMARY

The growth and development of macadamia seedlings and effects of applications of Paclobutrazol to seedlings and bearing trees were investigated.

Macadamia seedlings grown under controlled environmental conditions exhibited shoot flush growth with intervening periods of dormancy lasting one to two weeks. Flush patterns varied with plant age and between plants. Younger plants exhibited a more or less continuous flush pattern while older plants had longer intervening dormancy periods. Leaf growth involved leaf initiation, a rapid expansion phase and lignification. Stem extension and leaf growth appeared correlated and occurred simultaneously. Carbohydrate studies showed that plants had much higher leaf starch and sugars when they were in flush than when they were either dormant or were initiating growth.

Application of Paclobutrazol reduced shoot growth in both seedlings and bearing trees through reduced internode elongation. In both cases, growth was stimulated at the lowest levels of retardant. Paclobutrazol had no effect on individual leaf sizes but influenced total leaf area through increases in leaf numbers. In bearing trees,, Paclobutrazol had significant effects on yield and kernel recovery in the second year of application. Carbohydrate studies showed that in seedlings starch and sugar levels were increased with stimulated growth and were not affected at higher PBZ levels. However, in mature trees leaf sugars were lowest in plants with stimulated growth.

Applications of Paclobutrazol to seedlings resulted in the development of cluster roots. Cluster roots failed to develop in untreated plants but were evident in all treated plants. PBZ also increased root reducing and non-reducing sugar levels.

The implications of these results are discussed in relation to their possible causes. The potential for PBZ application in controlling growth in macadamia is also discussed.

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ABBREVIATIONS:

2,4,D 2, 4 - dichlorophenoxyacetic acid

ABA Abscisic acid

ACC 1-aminocyclopropane - 1 - carboxylic acid

ANOVA Analysis of variance

BA Benzyladenine

CCC Chlorocholine chloride

CEPA 2 chloroethylphosphonic acid

FRF Fruit removal force.

GA Gibberellin

$GA_3$ Gibberellin $A_3$

Glucose equiv. Glucose equivalents

IAA Indole - 3 - acetic acid

IBPGR International Board for Plant Genetic Resources

L.S.D. Least significant difference

NAA 1-naphthylacetic acid

PBZ, PP333 Paclobutrazol

S.D. Standard deviation

S.E.M. Standard error of means

S.E.D. Standard error of the differences between means

SADH Succinic acid - 2, 2- dimethyl hydrazide

TIBA 2,3,5 - triodobenzoic acid

TNSC Total non-structural carbohydrates

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# CHAPTER 1
# GENERAL INTRODUCTION

## 1.1 MACADAMIA

### 1.1.1. World importance

The macadamia is an evergreen subtropical tree which yields a nut of high nutrition and economic value. The nut is eaten fresh or roasted as a table nut or a cocktail snack accessory and ordinarily offered on gourmet shelves salted in jars or vacuum packed in foil. Due to its strong flavour it has also found uses in the confectionery trade and pieces of the kernel are used in biscuits and ice creams. High quality oil can be extracted from the kernel and used as a salad or cooking oil or in the manufacture of soaps and cosmetics. The nut also yields several by-products including press cake which is used as animal feed, the shell as a source of fuel, and husks make excellent plant growing media (Trochoulias, 1980).

Production is concentrated in subtropical areas. Currently, Hawaii dominates the world market, with over 70% of production while Australia supplies 10-15% of world trade. The rest is divided amongst several countries. Due to land limitations in Hawaii, no further expansion is expected while Australia is poised to become the world leader in macadamia production by year 2,000. Production estimates for 1987 are presented in Table 1.1.

### 1.1.2 Importance in Malawi

In Malawi, commercial interest in the macadamia arose in the early 70's. The hectarage has since steadily increased to over 2,000 hectares. Despite the relatively small hectarage, the crops' contribution to the country's economy is enormous. As it is largely an export crop, it contributes greatly to the country's foreign exchange earnings, mostly due to the high value the nuts command on the market. According to a report published by the Malawi Tree Nut Authority (TNA, 1991) the 1989

production of salable kernel amounted to 150 tonnes and returned MK3.18 million (MK4 = £1, MK=Malawi kwacha). Malawi possesses a competitive advantage over most other producers because of availability of labour and low land costs.

Table 1.1 World annual production (kernels) and distribution of macadamia nuts.

| Region | Country | Kernels (tonnes/year) |
|---|---|---|
| Africa | Kenya | 180 |
| | Malawi | 90 |
| | South Africa | 230 |
| | Zimbabwe | 50 |
| Asia | Australia | 1000 |
| Central America | Costa Rica | 180 |
| | Guatemala | 140 |
| USA | Hawaii | 4860 |
| Total | | 6550 |

Source: Vigden and Leeson (1987)

The nutritional contribution of macadamia, though often overlooked, is potentially quite significant. Malawi is basically a rural economy with over 50% of the population engaged in agriculture or agricultural-related enterprises (National Statistical Office, 1987). Most of the rural population survive on basic diets of starch and little protein. It would appear, therefore, that crops like macadamia could contribute to the nutritional status of the nation. Macadamia is largely an estate or plantation crop, the smallholder element is currently small with about 200 hectares ranging in size from less than 5 to 1,000 trees for each holding (TNA, 1991).

However, the potential seems very high especially in farming systems which involve interplantings.

Macadamia kernels are rich in unsaturated fatty acids (Cavalleto, Dela Cruz, Ross and Yamamoto, 1966) with the main oil constituents as oleic (59-67%), palmitoleic (19.1-22.1%) and palmitic (6.15-8.7%) acids. The major protein amino acids in macadamia nuts are arginine, glutamic acid and leucine. The overall composition of whole roasted kernels is shown in Table 1.2.

Table 1.2 Nutritional composition of Macadamia kernels.

| Component | g. 100g-1 |
|---|---|
| Oil | 78.2 |
| Carbohydrates | 10 |
| Proteins | 9.2 |
| Moisture | 1.5-2.5 |
| Potassium | 0.37 |
| Phosphorus | 0.17 |
| Magnesium | 0.12 |
| Calcium | $36 \times 10^{-3}$ |
| Sodium | $6.6 \times 10^{-3}$ |
| Iron | $1.8 \times 10^{-3}$ |
| Zinc | $1.4 \times 10^{-3}$ |
| Manganese | $0.38 \times 10^{-3}$ |
| Copper | $0.33 \times 10^{-3}$ |
| Niacin | $1.6 \times 10^{-3}$ |
| Thiamine | $0.22 \times 10^{-3}$ |
| Riboflavin | $0.12 \times 10^{-3}$ |

Source: Wenkham and Miller (1965)

### 1.1.3. Taxonomy

Macadamia belongs to the family Proteaceae, sub-family Grevillidae (Brown, 1984)). The genus Macadamia has ten tree and shrub species, six of which are native to Australia, three to New Caledonia in the South Pacific and one to Sulawesi in Indonesia (IBPGR, 1986). The edible nuts are produced by two species: M. integrifolia (Maiden and Betche), known as 'the smooth-shell' type and M. tetraphylla (L.A.S. Johnson), known as 'the rough-shell' type. Hybridization occurs freely between the two species and is fast becoming an important source of variability for clonal selection. The other species are mostly inedible with small and often bitter kernels due to the presence of cyanogenic glucosides (Malo and Campbell 1982). These include:

M. ternifolia (from Australia)
M. heyana "
M. prealta "
M. whelani "
M. francii (from New Caledonia, South Pacific)
M. roussellii "
M. veillandii "
M. hildebrandii (from Sulawesi, Indonesia)

Natural macadamia stands are highly variable in tree size, fruit size, shell thickness, yield and quality. Likewise, seedlings exhibit a wide range of morphological features indicating a heterozygous outbreeding nature.

### 1.1.4. Origin and distribution

The macadamia tree is native to the eastern coastal areas of Australia $23^0$ to $29^0$ South of the equator. M integrifolia occurs naturally only in southern Queensland $25^0$ to $28^0$ S and M. tetraphylla occurs only in the northernmost part of New South Wales and southernmost part of Queensland $28^0$ to $29^0$ S. Their natural ranges overlap in

southern Queensland where putative hybrids between them have been reported (Johnson, 1954; Smith, 1956). The first species of macadamia was collected in 1843 for the National Herbarium in Melbourne by a German explorer named Ludwig Leichhardt. However, it was not described botanically until 1857 by Baron Ferdinand von Mueller, a leading Australian Botanist, following a botanical expedition in Queensland. He named Macadamia ternifolia in honour of his friend Dr John Macadam who was also a distinguished philosopher (Rosengarten, 1984). The first commercial orchard was planted in 1888 near Lismore, New South Wales.

Although it was first domesticated in Australia in 1858, the promotion of macadamia as a commercial crop took place in Hawaii, where the first known introduction was made in about 1882 by H. Purvis (Ito, 1983a). The first attempt at commercial production was made in 1922, and in 1926 the plants were grafted successfully for the first time. In 1948 varietal status was awarded to five selected smooth shelled clones from the original seedlings. The crop has since spread to several countries in Central and South America, Asia and parts of East and Southern Africa.

### 1.1.5. Structure and habit

A healthy mature macadamia tree may attain a height of over 20m with a spread of 15m at 15 years of age. The canopy can be open or dense with upright or spreading forms and single or multiple stems. Plate 1.1 shows the structure of a healthy 10 year old M. integrifolia tree growing in Malawi. Mature leaves are sclerophyllous which enables them to resist collapse after loss of turgidity so that they do not show stress until it is extreme and irreversible.

Flowers are perfect and are borne on long racemes. The racemes are borne on two year old or older wood on a rachis 20 to 30 cm long. Plate 1.2 shows flower racemes on a bearing M. integrifolia tree during anthesis. Each raceme contains more than 200 perfect flowers (Ito, 1983b). Flowers are protandrous and also have a self-

Plate 1.1 Structure of a 10 year old tree of Macadamia integrifolia growing in Malawi.

Plate 1.2 Flower racemes on a bearing branch of a M. integrifolia tree. Each long raceme may have up to 300 flowers.

incompatibility mechanism, the extent of which varies between clones (Sedgley, Blesing and Vithanage, 1985). This encourages cross pollination. In addition, varying degrees of self-incompatibility have been reported (Sedgley, 1983) resulting from inhibition of pollen tube growth in the style.

Distinct structural differences exist between the two edible species. The leaves of M. integrifolia are characteristically borne in whorls of three at each node and are relatively spineless at the edges. The flowers are creamy white and the kernels white with up to 80% oil and 4% sugar (Malo and Campbell, 1982). It has an excellent flavour and quality which makes it suitable for processing. M. tetraphylla, on the other hand, has leaves borne in whorls of four to five per node with thorny, spined margins. The flowers are pink or creamy white and the kernels are darker in colour with a higher sugar content and are more variable in quality. This, coupled with low oil levels (67-75%), makes M.tetraphylla variable in texture and flavour when cooked (Hamilton, Ito and Chia, 1968) and hence unsuitable for processing. Most commercial clones are of M. integrifolia type. M. tetraphylla was mostly used as a rootstock until the introduction of clonal rootstocks a few years ago. Details in the major characters of the two species are listed in Table 1.3.

The macadamia fruit is a follicle with a husk (dull green fleshy pericarp) that opens along one suture line from the stalk to the distal end enclosing a single spherical seed with a very hard testa or shell. Fruits are borne in a cluster on a single raceme (Plate 1.3). The shell, which is either rough (M. tetraphylla) or smooth (M. integrifolia) (Plate 1.4), in turn encloses a white kernel which is the major economic product.

### 1.1.6. Genetics and breeding

Macadamia is a tetraploid genus with 2n=28 for M. integrifolia, M. tetraphylla and M. ternifolia (Brown, 1984). Polyploidy has been reported, with at least one clone of M. integrifolia having 2n=56 (IBPGR, 1986). The production of either polyploid or

Table 1.3 Principal characters of M. integrifolia and M. tetraphylla.

| Character | M. integrifolia | M. tetraphylla |
|---|---|---|
| Phyllotaxy | 3 leaves in nodal whorl seedlings may have only 2 some branches have 4 | 4 leaves in nodal whorl, seedlings have 2, some branches have 3 or 5. |
| Leaf attachment | petiolate | sessile or scarcely subsessile |
| Adult leaf shape | oblanceolate to obovate | oblanceolate |
| Adult leaf margin | generally entire, sometimes with 1-12 teeth on a side | numerous serrations ranging from 15-40 on a side, ocassional leaves have fewer than 15. |
| New growth | pale green, ocassionally with bronze tinging. | pink to red, ocassionally yellowish green due to lack of anthocyanin |
| Racemes | 10-30cm long with 100-300 flowers | 15-45 cm long with 100-300 flowers |
| Pericarp | bright clear green due to nearly glaborous condition, often fails to dehisce when fruit is still on the tree. | grayish-green due to fairly dense white pubescence, dehisces fully on tree before fruit drops. |
| Seed size | 12-32mm | 12-38mm |
| Seed shape | commonly spherical | commonly fusiform, some nearly spherical. |
| Seed surface | generally smooth rarely with slight pebbling. | generally pebbled, rarely smooth. |
| Kernel | sweet, highly palatable. | sweet, highly palatable. |

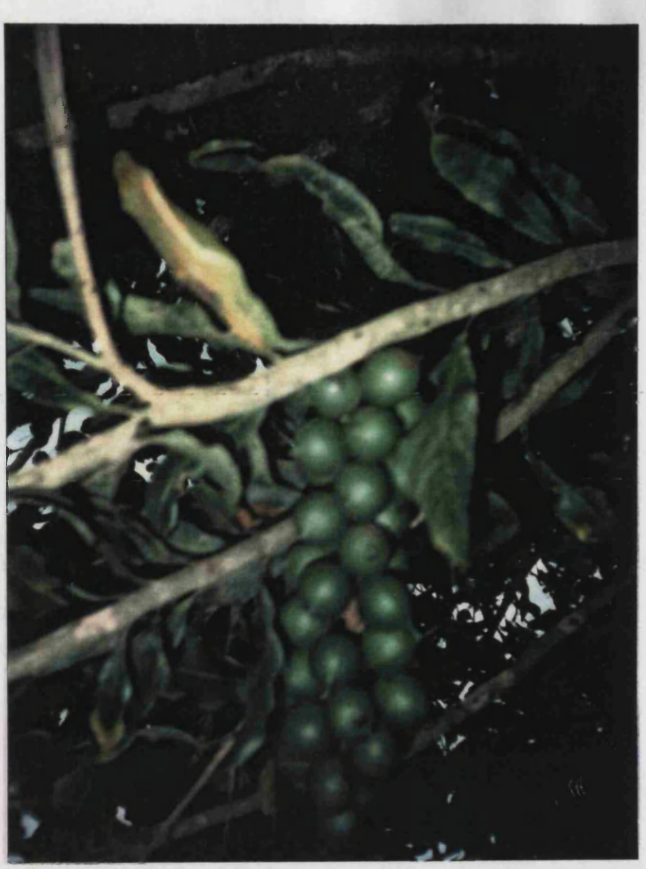


Plate 1.3 A cluster of nuts formed on ~~from~~ a single raceme. Most racemes normally end up with 0 to 5 nuts at maturity.


Plate 1.4 Inshell nuts of **(a)** M. integrifolia and **(b)** M. tetraphylla. Note the respective rough and smooth shells in the two species. **(c)** Macadamia kernels.

aneuploid strains could lead to the improvement of features not previously considered. Interspecific hybrids of macadamia are found naturally and as a result of close planting in orchards (Ito, 1983b). Due to lack of knowledge as to the inheritance of particular traits, and lack of inbred lines of known characteristics, it is difficult to carry out controlled breeding and testing. However, the potential for selection from known good parent crosses is high given the heterozygous nature of the tree, although large plant populations may be required for selection and a time frame of up to 15 years may be necessary.

Most of the commercial clones have been developed in Hawaii where selection began in 1934 and about 100,000 seedlings have been examined so far (Ito, 1983a). This has resulted in the development of several clones which currently form the basis of the world macadamia industry. Most Hawaiian clones are numbered after the tree from which they originated. Some of the earlier selections such as 246 (Keauhou), 333 (Ikaika), 508 (Kakea), and 660 (Keaau) have been superseded by more condition-specific clones such as 344 (Kau) (for difficult conditions), 741 (Mauka) (for high elevations), and 800 (Makai) (for low elevations).

The Australian macadamia industry is developing some locally adapted clones (Bell, 1984; Winks, Gallagher and Lanhan 1988) from both local sources as well as introduced material. Selection is mostly based on nut quality, kernel recovery and yield. Other factors including climatic adaptation, processing qualities and pest and disease resistance are also taken into consideration (Nissen and Williams, 1978)

### 1.1.7. Ecology and climate

The macadamia tree is generally adapted to a subtropical climate and although it originates from a small ecological zone it has adapted well even in areas quite different from its area of origin. For example, due to genetic selection in Hawaii the macadamia gives a superior performance there compared to Australia where it

originated from. Macadamia trees grow well from altitudes of below 150m in Hawaii (Shigeura, 1981) to altitudes of 1600m in Costa Rica (Cull and Trochoulias, 1982)).

Temperature seems a very important factor for both floral initiation and vegetative growth. Floral initiation in Australia occurs under shortening days with night temperatures of $11^0$ to $15^0$C (Stephenson and Gallagher, 1986) while in the more tropical climate of Hawaii it occurs at higher temperatures ($15^0$ to $21^0$C) (Nagao and Sakai, 1985). Temperatures have a big influence on vegetative growth (Stephenson, Cull and Stock, 1986). Allan and De Jagger (1978) consider $23^0$ to $24^0$C as optimum for macadamia growth. Trochoulias and Lahav (1983) reported that macadamia growth was restricted to the temperature range $15^0$ to $30^0$C with an optimum for leaf area growth and dry matter production at $20^0$ to $25^0$C. Temperatures above $30^0$ result in much higher respiration rates and, therefore, affect the accumulation of oils in the kernel and also increase premature nut abcission (Stephenson and Gallagher, 1986). Temperatures below $15^0$C are not conducive to flowering and ultimately depress yield (Shigeura, 1981).

Macadamias grow better in areas with at least 1,000 mm rainfall (Cull and Trochoulias, 1982), although no yield responses to irrigation have been obtained in Australia (Trochoulias, 1988). In Hawaii, however, irrigated trees have shown higher yields primarily as a result of the greater number of nuts produced rather than their size (Awada, Warner and Watanabe, 1967). Mild frosts and droughts adversely affect yield (Hamilton, Ito and Chia, 1968). The tree thrives on a wide range of soils especially those which are well drained with a pH of about 6.5 (Allan, 1972). A high water table resulting in a waterlogged root zone is detrimental to root development and restricts tree growth.

### 1.1.8. Phenology

Macadamia is an evergreen tree and retains the bulk of its leaves throughout the year. Some old leaves are shed over summer. Vegetative growth in mature trees is achieved through a cyclic pattern of vegetative flushes. Shoots grow for a time producing leaves and buds at each node, then cease growth while the leaves and stem that have recently been produced gradually become hardened. In Malawi flushes of growth occur several times in a year with peaks in late summer and early spring. Reproductive growth, involving flowering, nut set, nut fill and maturity takes place more or less concurrently with vegetative growth.

#### 1.1.8.1. Vegetative growth

Vegetative growth is achieved through a series of growth flushes depending on location. In most areas of Malawi trees undergo several flushes in a year but flushing peaks occur in late summer/early autumn (Jan-March) and early spring (Aug-Sept). In Australia, the occurrence of a dominant summer flush and a smaller spring flush has been reported (Stephenson and Cull, 1986). In general, the major flushes are restricted to times when temperatures are relatively mild and are unlikely to occur at temperatures lower than $10^0$C and above the threshold temperature of $30^0$C (Allan, 1983). Low winter temperatures are effective in restricting growth and spring flushes occur only after mean monthly temperatures rise above $10^0$C. In Australia the late summer/autumn flushes occur towards the end of the upper threshold temperature (Stephenson, Cull and Stock, 1986). The availability of nutrients also influences flushing patterns. Significantly greater spring and autumn flushes have been reported in Australia following nitrogen application (Stephenson and Gallagher, 1983).

#### 1.1.8.2. Reproductive development

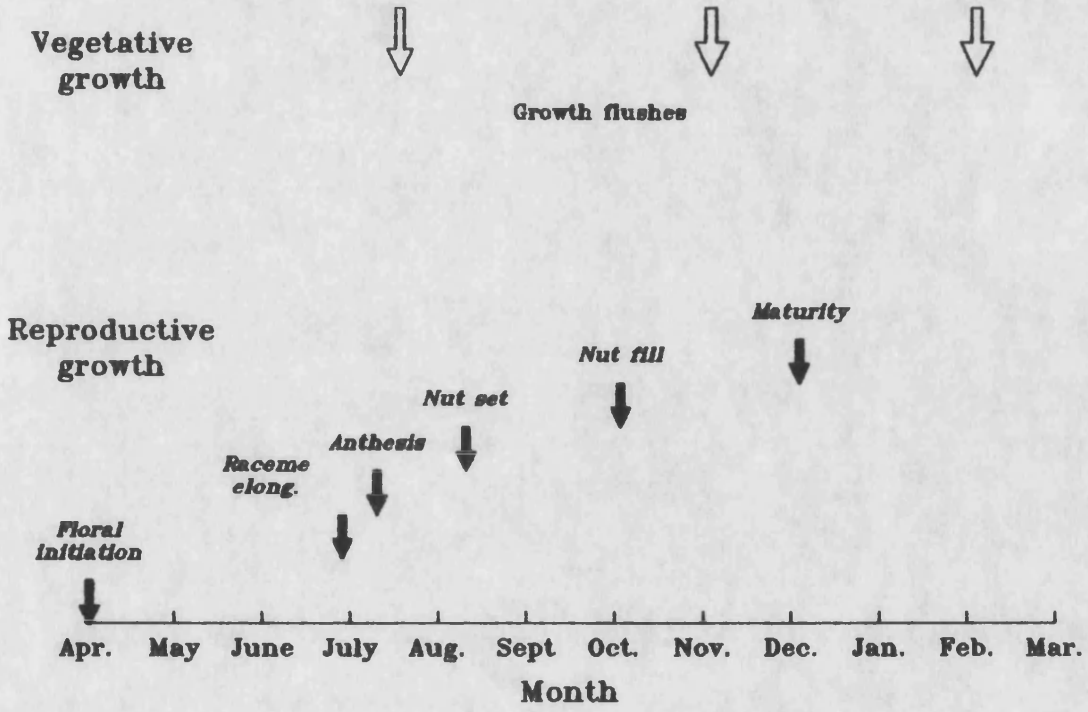
In Malawi, flowering occurs over a period of five months from early winter through spring (April to September) and involves floral initiation, raceme elongation and anthesis. Floral buds initiate during the shortening days of autumn (April/May), a

period of low temperatures and declining solar radiation. There then follows a period of 'dormancy' which is rather a period of low growth activity due to unfavourable winter conditions, particularly low temperatures, reduced radiation, and low precipitation. In early spring (August) the elongation of racemes from the floral buds takes place. Rising temperatures and increased solar radiation results in rapid elongation and differentiation of the floret. Florets enlarge, become tubular in shape with an enlarged cap and are attached to the raceme by a short pedicel. A week before anthesis, the style elongates and anthers dehisce a few days later (Moncur, Stephenson and Trochoulias, 1985). Fig. 1.1 briefly summarises the timing of vegetative and reproductive development in relation to each other under conditions of Southern Malawi.

Anthesis occurs throughout spring. The major part of anthesis is reported to occur in the early morning although some flowers on a single raceme have been observed opening throughout the day in Hawaii (Ito, Eyre and Cabral, 1983). Since anthers dehisce before anthesis, pollination is ensured by outcrossing. According to Sedgley (1981) pollination is mostly carried out by bees (Apis mellifera), the ovary contains two ovules at anthesis but only one is normally fertilised. The unfertilised ovule eventually becomes crushed by the fertilised one. Fluorescence microscopy has shown that the growth of self pollen tubes becomes arrested in the style (Sedgley, 1983). The pattern appears to be characteristic of gametophytic incompatibility where the genotype of the individual microspore and of the female parent determine the reaction. Inhibited pollen tubes have swollen tips and appear to discharge their contents through a subterminal pore.

In compatible pollinations, fertilisation occurs 48 to 72 hours after anthesis (Ito, 1980). Fruit growth following fertilisation occurs in a single sigmoidal pattern (Sakai and Nagao, 1984). A rapid increase in fruit size begins 2 to 3 weeks after anthesis and continues until 14 to 16 weeks later. Nut growth is enhanced at temperatures of $25^0$ to

**Fig. 1.1 Annual pattern of vegetative and reproductive growth phases in mature macadamia trees in southern Malawi.**



$30^0$C (Stephenson and Gallagher, 1986) but extreme temperatures during the later stages of nut development adversely affect nut growth. The embryo, which develops into the kernel, then develops and accumulates oil. This is followed by hardening of the shell, which turns brown on the inside as a sign of maturity. Fully mature nuts dehisce and fall to the ground from where they are picked.

During all this period there is continuous abcission of flowers and nuts which occurs in 3 stages (Sakai and Nagao, 1984) involving: abcission of about 90% of all flowers in the first 2 weeks after anthesis; a rapid abcission of 80% of the initial nut set 3 to 8 weeks after anthesis; and gradual abcission of larger immature nuts throughout development.

### 1.2. Excessive Vegetative Growth

Despite enormous interest and success with macadamia in Malawi, there are several production problems. These relate to low productivity arising from poor management, adverse environmental effects, pests and diseases and an overall insufficient knowledge of the crop.

One problem of special interest and especially relevant to the present research project is that of excessive vegetative growth without related increases in nut production. This has led to large expenditures on farm inputs such as fertilisers, pesticides, and labour to manage large and relatively unproductive trees. In addition, trees have to be spaced wide apart giving low plant populations (100 trees/ha), hence under-utilising land area. Tree size control is a problem with macadamias particularly in Malawi, where trees apparently grow larger than in Australia or Hawaii. The relatively large tree sizes are as a result of the occurrence of more growth flushes per season as compared to Australia where only one major flush occurs (Stephenson and Cull, 1986b). Since the trees remain in production for up to 50 years and grow to over 15m

in canopy diameter, thinning out becomes necessary. However, this brings about a decline in orchard productivity.

The phenology of the macadamia in Malawi (Fig. 1.1) indicates that vegetative growth flushes coincide with reproductive growth. For example the spring flush occurs during nut set. There is evidence to suggest competition for assimilates which results in low productivity during this period. Stephenson, Cull and Stock (1986) reported that under Australian conditions new growth flushes depleted accumulated reserves. They noted that although vegetative growth was needed to provide the branch framework for future flower buds, and assimilates to support future nut growth, it competed with and reduced the current nut crop.

One factor in the reduction of cropping is competition for nutrients. Stephenson, Cull, Mayer, Price and Stock (1986) reported an accumulation of leaf N, P, and K during dormant periods in autumn and winter and a decline in spring and summer in macadamia under Australian conditions. The decline was closely associated with vegetative flushes, suggesting that elements were mobilised from the mature leaves to support new growth. This may result in competition between the developing flush leaves and the developing nuts. Since the rapidly expanding young leaves are a stronger sink than the developing fruit (Allan, 1983), the excessive vegetative growth is, therefore, achieved at the expense of reproductive growth. In support of this, further research has shown that the absence of flushes during early summer when nut set and oil accumulation is occurring results in improved yields (Stephenson and Cull, 1986a). Similar occurrences have been reported in apples where lack of shoot competition after flowering favoured fruit set and development (Pereira, 1975).

### 1.2.1. Controlling vegetative growth

Several techniques have been used in tree crops to control growth and help regulate the balance between vegetative and reproductive growth. Such techniques include:

use of genetic dwarfs, dwarfing rootstocks, pruning/hedging and irrigation, and the application of plant growth regulators.

1.2.1.1. Genetic dwarfs

Genetically dwarfed tree cultivars offer the possibility of increasing tree crop productivity through genetically altered patterns of dry matter allocation. Genetic dwarfs of peach cultivars have been found to allocate a larger proportion of their above-ground dry matter to leaves, fine branches, and fruit and a smaller proportion to the larger wood than do normal trees. Compact genetic dwarf canopies have larger amounts of leaf area and leaf nitrogen per unit of canopy volume than standard trees (DeJong and Doyle, 1984).

Genetic dwarf cultivars are obtained through breeding (i.e crossing) and by ionising radiation. In some cases mutants occur spontaneously in orchards. The occurrence of growth type mutations of MacIntosh apples in several orchards has been reported (Looney and Lane, 1984). Some dwarf commercial cultivars obtained by cross pollination and ionising radiations have been reported in sweet cherry and peach (Fideghelli, Della Strada and Quarta, 1984) and blue berry (Drapper, Chandler and Galletta, 1984). Genetic dwarfs offer the opportunity to increase early yields by enabling planting at higher densities and reducing the production costs incurred in pruning and managing large trees. They do not, however, occur naturally in most species and even when they do, it may be difficult to combine the unique growth habit with other desirable characteristics.

1.2.1.2. Dwarfing rootstocks

The use of rootstock cultivars which have a dwarfing effect on the scion has gained wide popularity in modern orchard management. This is particularly so in apples where a number of dwarfing rootstock cultivars have been developed including M9, M26, and M27. Chalmers, Mitchell and Jerie (1984) noted that the growth rate or

vigour of the vegetative portion of the plant was directly correlated with the growth rate of the roots and that the roots of dwarfing rootstocks had slower growth rates, determined genetically, which limited scion growth.

The use of interstocks to induce dwarfing on a tree growing on a vigorous rootstock is also widely used (Rogers and Beakbane, 1957; Parry and Rogers, 1968). Use of dwarfing rootstocks or inter stocks brings its own problems. Information on appropriate rootstock/scion combinations for various environmental conditions is lacking and the degree of precocity induced by use of dwarfing or semi-dwarfing rootstocks is not always adequate for the requirements of the system (Luckwill 1978). In some instances the use of dwarfing rootstocks can introduce other problems concerned with root anchorage, disease resistance or physiological disorders such as 'Cox's disease'.

1.2.1.3. Pruning/hedging and irrigation

Apart from getting rid of dead wood and reducing mutual competition for light, pruning has a dwarfing effect on the tree. Pruning results in an immediate reduction of tree size and the effect of tissue removal, reduction of leaf, shoot, or root volumes and carbohydrate and nutrient reserves contained in the removed parts may directly limit potential growth (Stiles, 1984). In addition changes in the rates of production or balance of endogenous growth regulators, carbohydrate mobilisation and distribution indirectly alters growth potential.

Several methods of pruning including central leader, spindle bush, and palmette have been tested with little success on macadamia. Heavy pruning has been found to be detrimental to yield (Trochoulias, 1983). Most recommendations currently centre on a basic tree structure which ensures freedom from poor crotch angles in the first three years. No further tree structural training is done except for the removal of damaged, misshapen or protruding limbs (Cull, Stephenson and Winks, 1983)

Timing of pruning seems to be important in developing a balance between reproductive and vegetative growth. Summer pruning has been reported to control vigour while promoting flower set on peaches in spring (Erez, 1984). However, some trees such as apples recover well from pruning as they have an inherent ability to produce compensatory growth in response to alteration in root:shoot ratios (Myers and Feree, 1984).

Hedging has also been used to reduce vigour of mature trees and improve fruit qualities (Emerson and Hayden, 1984). In some cases, however, hedging has resulted in yield depression, reducing fruitfulness by half compared to summer pruning in peach (Chalmers, Mitchel and van Heek, 1981).

Irrigation can also be used to control the balance between vegetative and reproductive growth. In the phenology of most deciduous perennial tree fruit crops, vegetative growth is followed by fruit growth. Hence a simple irrigation strategy can suppress one form of growth without affecting another. Chalmers et. al. (1984) found that vegetative growth of peach and pear trees was reduced by 80% and 70% respectively when daily water allocation was reduced to 1/8 and 1/4 of class A evaporation pan level during fruit growth. Fruit size was not affected but a 30% increase in final fruit yield was obtained. The yield increase resulted from the fact that fruit growth was stimulated after irrigation was raised.

Pruning, hedging and other cultural practices aimed at regulating tree size are essential for effective tree management. They are, however, labour intensive, costly and do require a lot of time. In most cases, especially in the tropics, these practices result in heavy regrowth which may increase canopy size even more.

1.2.1.4. Plant growth regulators

A clear distinction ought to be made between plant hormones and plant growth regulators or growth substances. Moore (1989) has defined or rather described a plant hormone as 'an organic substance other than a nutrient, active in very minute amounts or concentrations, which is formed in certain parts of the plant and which usually is translocated to other sites where it evokes specific biochemical, physiological and/or morphological responses'. He has further described a growth regulator or growth substance as 'an organic compound, other than a nutrient, which in small amounts promotes, inhibits or qualitatively modifies growth and development'. Hence all hormones are plant growth substances but the converse is not true. There are many purely synthetic compounds which qualify as growth regulators but which are not hormones.

Plant hormones are involved in many aspects of growth and play a central role in the correlation and coordination of growth. Thus growth of fruit tissues is dependent on the supply of hormones from the developing seed, and leaves are dependent upon cytokinins from the roots. Many plant growth responses to environmental stimuli such as tropisms and nastic responses are, apparently, mediated through the hormone system. Environmental factors often exert inductive effects by evoking changes in hormone metabolism and distribution within the plant. In addition, hormones also are the principal agents which otherwise regulate expression of the intrinsic genetic potential of plants.

In a particular plant organ, locally produced hormones and those supplied as signals from elsewhere interact. It is the resultant of this hormonal balance which determines the further development of the organ. This balance leads to the inhibition or promotion of germination, the formation of lateral shoots and roots, and the induction of flowers among many others. Bruinsma (1985) noted that hormones accomplished

this through their ability to interfere in a highly specific way with the expression of the genetic information of the cell to initiate the expression of a particular part of the genome and to suppress another part. It is not clear to what extent control of growth and development by a hormone is correlated with the changes in concentration of the hormone or changes in sensitivity of the tissue to it. The latter concept has been emphasized by Trewavas (1981) due to a lack of correlation between hormone concentration measured in tissues and responses of the tissues.

The commonly recognised classes of plant hormones are the auxins, giberellins, cytokinins, ethylene, and abscisic acid. Auxins are formed in the overground plant parts and promote growth, especially cell division and elongation. They play a role in the development of stems, fruits and seeds. Auxins are also effective in the induction of adventitious and lateral roots. Gibberellins promote germination and elongation of shoot growth. They also improve the size of flowers and fruit set (Goldwin, 1985). Not all of the over 60 gibberellins are hormones themselves, the majority are probably intermediates of biosynthetic and other metabolic pathways. Cytokinins are root produced regulators which promote cell division and anabolic metabolism and postpone senescence symptoms (Bruinsma, 1985). Ethylene has different effect in growing and in mature organs; it inhibits growth of young organs and promotes abscission and senescence of fully grown organs including yellowing of leaves, fading of flowers and ripening of fruit (Bruinsma, 1983). Abscisins inhibit growth in young tissues and may play a role in tropistic curvatures by locally restraining cell elongation in stems and roots (Pilet, 1983). They also promote senescence and abscission of mature organs and induce dormancy in seeds and buds (Addicot, 1983). The most prominent abscisin, abscisic acid is too labile to be applicable in practice.

The production of synthetic hormone derivatives has resulted in the availability of compounds with wide potential for use in agriculture. According to Bruinsma (1980) there are two ways in which exogenous plant growth regulators can interfere with the

endogenous hormonal pattern. Firstly they can interfere with the biosynthesis, translocation or metabolic conversion of plant hormones; and secondly they can replace or supplement phytohormones when the level of the latter is sub-optimal. Examples include shortage of ethylene which can be overcome with ethephon or by stimulation of its biosynthesis with auxin or ACC (1 - aminocyclopropane - carboxylic acid), the compound from which ethylene is formed (Bruinsma, 1983).

In most cases synthetic regulators are more effective than the endogenous hormones (Research reviewed by Bruinsma, 1980). For example, synthetic auxins are more stable and because of their similarity in aspects of chemical structure to the natural hormone the synthetics are accepted by auxin binding sites and are thus able to exert auxin effects. Some of them act very strongly and lead to growth aberrations e.g 2,4 D (Blackman, 1945). An overdose of auxin may lead to formation of ethylene. Ethylene releasing chemicals such as ethephon have been synthesized and can be applied as spray or a soil drench. They decompose within the plant releasing ethylene. On the other hand, inhibitors of ethylene synthesis are used to prolong the shelf life of harvested commodities. (Bruinsma, 1983)

The successful applications of hormones, their derivatives, or inhibitors in agriculture and horticulture may be grouped as follows;

1. To achieve an increase in the rate of growth.
2. To act as a growth trigger ie stimulate some aspect of growth.
3. To change the balance of growth and partition of assimilates within the plant.
4. Achieve some qualitative switch in plant development e.g. induction of flowering, rooting of cuttings.
5. Accelerate or delay senescence.
6. Modify metabolism to increase the production of some specific product.

Recently there has been much interest in the use of synthetic growth regulators in order to influence production. Of special interest are those regulators which inhibit or retard tree growth and development. According to Dicks (1979), plant growth retardants are 'synthetic, organic compounds which, when applied to a responsive plant, reduce the rate of stem elongation by inhibiting subapical meristem activity, normally without exerting substantial effects on leaf production and development or inducing other growth malformations'. Growth retardants , therefore, offer a means of controlling vegetative growth and improving the partitioning of assimilates between fruits and the rest of the tree. Their use can aid management and improve productivity of trees planted at higher densities than normal.

Cathey (1964) defined 'growth retardants' as chemicals which slow down cell division and elongation in shoot tissues and so regulate plant height without other formative effects. Examples are chlormequat (chlorocholine chloride), daminozide (succinic acid-2,2-dimethyl hydrazide), and phosphon-D (2,4-dichlorobenzyl - tributyl phosphonium chloride). 'Growth inhibitors' on the other hand completely inhibit growth in the shoot meristems and at high concentrations suppress all growth. Several regulants are now in use to control plant height through the following mechanisms (Sachs and Hacket, 1972);

(a) Terminal bud destruction: Maleic hydrazide (1,2-dihydro - 3,6-pyridazinedione), TIBA (2,3,5-triodobenzoic acid), and ethephon (2-chloroethyl phosphonic acid) act by killing the terminal bud or by causing severe disruptions in the functions of the apical meristem. The compounds alter geotropic responses and cause bud break or early leaf abscission. These inhibitors cannot be used where normal leaf and flower initiation and development are necessary.

(b) Internode elongation inhibition: Daminozide (SADH), paclobutrazol and chlormequat (CCC) inhibit internode elongation without disruption of the apical

meristem. In this case the influence of retardants on the shoot system is nearly completely prevented by simultaneous application of gibberellic acid.

(c) Reduced apical control: Resulting from simultaneous growth of too many shoot axes. These retardants may enhance flowering and appearance but are less effective than the terminal bud inhibitors, particularly on woody spp.

A certain amount of research has been conducted on the use of growth retardants on tree crops. Successful results have been reported with CEPA (phosphon D)/SADH combinations on apples (Fletcher and Kirkwood, 1982) and sweet cherry (Sansavini, 1984); mefluidide on peach (Arnold, Aldrich and Martin, 1983; Coston and Gambrell, 1984); paclobutrazol (Williams and Edgerton, 1983; Shearing and Jones, 1986; Webster and Quinlan, 1986) and daminozide (Edgerton and Hoffman, 1965; Halfacre, Barden and Rollins, 1968; Batjer, Williams and Martin, 1973) on a wide range of plants.

It is apparent that growth retardants could find a lot of use in the control of growth in macadamia. Timely and effective control of the vigorous vegetative growth could lead to the development of a compact tree which is more easy and cheaper to manage, with possible yield increases resulting from either increased production per tree or in creases in plant population.

### 1.1.3 AIMS OF THIS STUDY

In Malawi there is a need to improve the cropping of macadamia trees whilst simultaneously maintaining continued growth. One way of achieving this could be by regulating the balance between vegetative and reproductive growth i.e controlling excessive vegetative growth to improve reproductive growth. This project was carried out to investigate the growth of macadamia plants and to explore the use of paclobutrazol in controlling excessive vegetative growth.

To achieve this, a broad investigation was conducted which examined various aspects of seedling and mature tree growth and how these were affected by the chemical treatments. Firstly studies on the vegetative growth patterns in seedlings were conducted to monitor various phases of growth and patterns of stem and leaf growth and development. Secondly, changes in leaf and root carbohydrate levels were monitored at each phase of growth with the aim of identifying stages at which accumulation or depletion of leaf and root reserves occurred as indicators of demand and sink activity. Consequently, paclobutrazol was applied to seedlings and mature trees in a series of experiments. Vegetative growth, yield and quality were monitored in all experiments. In addition, leaf and root carbohydrate levels were monitored and related to growth phases and responses to paclobutrazol application. Studies were also conducted specifically on the effects of paclobutrazol on root growth and carbohydrate distribution in a quest to improve the poor root growth in macadamia plants.

# CHAPTER 2

# GENERAL MATERIALS AND METHODS

While experimental designs and some methods varied depending on type of study, the following methods were uniform wherever used in the project:

## 2.1. PLANT MATERIALS AND GROWING CONDITIONS

Nuts, from several trees of Macadamia integrifolia, were imported from Bvumbwe Research Station, Malawi in September 1988, April 1989, and February 1990. Upon arrival they were soaked in water for 24 hours then sown in sand (less than 3mm size) in seed trays placed on the glasshouse bench at temperatures of $21(\pm 3)^0$C minimum and $27(\pm 3)^0$C maximum with 16 hours supplementary illumination.

Due to poor development of seedlings potted in 100% compost, an observation trial was set up in April 1989 to investigate growth in mixtures of various proportions of Perlite (Silvaperl standard grade, Silvaperl Ltd, Gainsborough, Lincolnshire, UK.); bark (Cambark 100 Grade, Camland Products Ltd, Fordham, Cambs. UK); and compost (Levington C2 compost, Fisons PLC, Ipswich, Suffolk, UK) (Table 2.1). Seedlings were grown in 2 litre plastic pots. Two plants wee tested for each mixture. After 3 months, plant roots were cleaned of all soil and a visual analysis made on foliage appearance and vigour, and root structure. Shoots and roots were then oven-dried at $105^0$C for 24 hours and weighed.

Results on visual observation of plant vigour (Table 2.2) showed that mixtures with higher proportions of Cambark and Perlite resulted in vigorous seedlings with good root systems. In particular, combinations in the ratio 2:4:3 by volume C2 compost: Cambark: Perlite respectively resulted in the best growth including vigorous shoots and lots of healthy fibrous roots. This mixture was, therefore, used for potting seedlings in all subsequent studies.

Table 2.1 Composition of various mixtures and proportions of compost, cambark and perlite.

| Mixture | Proportions | | |
|---|---|---|---|
| | C2 compost | Cambark | Perlite |
| 1 | 6 | 2 | 1 |
| 2 | 4 | 2 | 3 |
| 3 | 4 | 4 | 1 |
| 4 | 4 | 3 | 2 |
| 5 | 2 | 6 | 1 |
| 6 | 2 | 4 | 3 |
| 7 | 3 | 4 | 2 |
| 8 | 2 | 3 | 4 |

Table 2.2 Visual appearance and vigour of foliage and roots of seedlings grown in various mixtures.

| Mixture | Foliage | roots |
|---|---|---|
| 1 | yellow and poorly | non-fibrous, no new roots |
| 2 | slightly yellow | many large roots |
| 3 | yellow and poorly | very few roots |
| 4 | green | many roots |
| 5 | green and vigorous | many fibrous roots |
| 6 | very green and vigorous | abundant fibrous roots |
| 7 | yellowish | heavy rooting |
| 8 | green | heavy rooting |

Ficote 140 (Fisons PLC, Ipswich, Suffolk, UK), a slow release fertiliser was incorporated in the compost mixture at the rate of 3kg/$m^3$. The seedlings were then grown in either of two environments:

(a). Environmental growth cabinet (model SAXIL, R. K. saxton Ltd, Bredbury, Cheshire, UK) at $25^0$C day and $17^0$C night temperatures. Supplementary light was provided (125 watts/$m^2$) for 12 hours per day. Relative humidity was not controlled.

(b). Glasshouse at 21($\pm$3)$^0$C minimum and 27($\pm$3)$^0$C maximum day temperatures, 16 hour photoperiod and uncontrolled relative humidity. Supplementary light (133 watts/$m^2$) was provided by high pressure sodium lamps. In summer the glasshouse was sprayed with Coolglass (Pan Britannia Industries Ltd, Waltham Cross, Herts., UK), a glass shading material to protect plants against excessive radiation.

Plants were watered twice weekly, and received a weekly nutrient solution containing 500ppm nitrogen and potassium in the form of 'Vitafeed 101' (Vitax ltd., Lancashire, UK) which contains 26% N, and 26% $K_2O$.

## 2.2. LEAF AREA DETERMINATIONS

### 2.2.1. Introduction

The literature contains references to many methods for the determination of leaf areas under laboratory conditions. These include photometric (Maggs, 1957) photoelectric (Bolas and Melville, 1933) and various other techniques such as tracing, shadow-graphing, and the use of planimeters (Kvet and Marshall, 1971). Most of these involve excision of the leaves and are therefore destructive.

Destructive techniques could not be used here as most of the studies involved monitoring of leaf area development over a period of time. Numerous attempts have been made to develop reasonably satisfactory methods of estimating leaf area on intact plants. A large number of these involve use of ratios and regression estimators by using easily measured leaf parameters such as length, width, dry weight which are then related to respective areas. Such methods have been used in tea (Fordham and Holgate, 1972), capsicum (Ray and Singh, 1989), grapes (Sparks, 1966; Manivel and Weaver, 1974), and macadamia (Cormack and Bate, 1975; Kobayashi and Uenten, 1984). These techniques, however, tend to be specific to varieties and species.

### 2.2.2. Determinations

Two methods of leaf area estimation were tested in order to determine the most accurate estimates

#### 2.2.2.1. Shadow-graphing (Kvet and Marshall, 1971)

Intact leaves were pinned to orange blueprint paper on the lower surface and clear acetate paper on the upper surface and subjected to intense light provided by one 275W and two 40W light bulbs for a few seconds until the orange colour disappeared. The blueprint paper was then removed and placed in a desiccator containing a beaker of liquid ammonia to fix the image imprinted by the leaf margins. The paper was cut

along the margins and passed through a portable leaf area meter (LI-COR LI 3000, LAMBDA Instruments Corp. Lincoln, Nebraska, USA)

This method was rather complicated and involved moving the plants in and out of the glasshouse or cabinet. Exposure of leaves to intense light and heat led to wilting in most non-hardened leaves, and accurate tracing of the serrated leaf margins proved rather difficult.

2.2.2.2 Estimation by calibrated regressions (Kobayashi and Uenten, 1984)

A random sample of 61 undamaged leaves of all sizes and ages was used for calibration. Leaves were excised from randomly selected plants and two measurements were made on each leaf: (a) The length from the point of attachment of leaf margin to the petiole (petiolar sinus) to the leaf tip, and (b) the leaf width at its maximum. The leaves were then passed through a portable leaf area meter. The relationship between the measured parameters and leaf area was examined by means of a stepwise regression analysis using MINITAB statistical package in a GOULD computer system. Regressions were determined with leaf area as the dependent variable. After testing several regressions including those involving transformations into squares, logs, and roots, the line of best fit (Fig. 1.1 and Table 2.3) was found with the following regression formula:-

$$\text{Log. Area} = -.0363 + .835(\log. L) + 1.09(\log. W)$$

$$\text{Leaf area (sq.cm)} = .964L^{0.835} * W^{1.09}$$

Where L is the length (cm) from petiolar sinus to the tip, and W is the leaf width(cm) at the widest point.

This method was adopted. Measurements of length and width were routinely made on intact leaves of all experimental plants. The values were then used in the conversion formula to derive estimates of leaf area.

Fig. 2.1 Leaf area calibration: Measured (leaf area meter) and calculated (length and width) leaf area relationships.

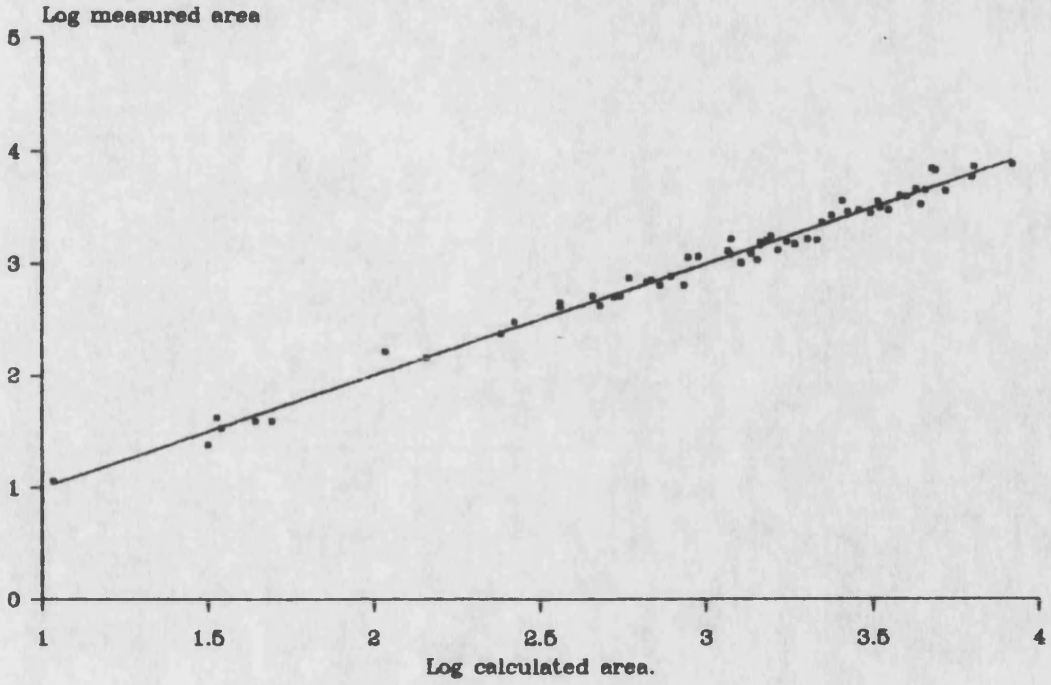


Table 2.3. Analysis of variance for the regression of leaf area on leaf length and width from 61 leaves of macadamia seedlings.

| Predictor | Coefficient | St. Deviation | t-ratio |
|---|---|---|---|
| Constant | -0.0363 | 0.09763 | -0.37 |
| Log. length | 0.8350 | 0.05187 | 16.10 |
| Log. width | 1.0898 | 0.05242 | 20.79 |

S = .07832 $R^2$ = 98.6% $R^2$ (adjusted) = 98.6%

Analysis of variance

| Source | DF | SS | MS | |
|---|---|---|---|---|
| Regression | 2 | 25.237 | 12.618 | ** |
| Error | 58 | 0.356 | 0.006 | |
| Total | 60 | 25.593 | | |

## 2.3. Carbohydrate Determinations

### 2.3.1. Introduction

Numerous enzymatic, chromatographic or other methods are available for the analysis of specific carbohydrates and related substances found in plant materials. Some methods are specific for reducing sugars (glucose and fructose), non-reducing sugars (sucrose and fructosans), and starch, while others hydrolyse all sugars including celluloses and hemicelluloses hence giving an overestimation of available sugars.

Several methods were tested to identify a suitable, simple but specific assay technique for reducing sugars, non-reducing sugars, and starch. Some techniques were found to be unsuitable as they were non-specific and gave total non-structural carbohydrates (daSilveira, Teles and Stall, 1978); others overestimated the available sugars by hydrolysis of celluloses and hemicelluloses (Yemm and Willis, 1954), others were rather complicated and required extensive time, reagents and glassware.

The Somogyi-Nelson method (Nelson, 1944; Somogyi, 1945) was found to be the most suitable for determination of reducing sugars. It was found to be relatively simple, specific and more efficient as it uses copper to oxidise the sugars. Copper reagents have been reported to oxidise sugars more selectively than iron (ferricyanide) solutions (Somogyi, 1945).

Non-reducing sugars were hydrolysed by invertase as per Wood (1984), and starch hydrolysed by a glucoamylase as per Thievend, Mercier and Guilbot, (1972), both yielding reducing sugars which were then determined by the Somogyi-Nelson colorimetric technique.

2.3.2.. **Test Samples**

2.3.2.1.. Sample preparation

Leaf and root samples were dried in a forced draught oven at $95^0$C for 1 hour followed by 4 hours at $70^0$C (Cormack and Bate, 1976). The dry matter was then shredded in a blender (Pulsematic Osterizer, manufactured by Oster Corp., Milwaukee, Wisconsin, USA) before being ground in a mill (Glen Creston Ltd, London, UK) fitted with a 1mm sieve. Samples of 200-500 mg were used for carbohydrate extraction.

2.3.2.2. Extraction

Samples were covered with 50ml 80% alcohol and warmed in a water bath (85 $\pm 2^0$C) for 1 hour. The solution was cooled and filtered through a Whatman No 4. qualitative filter. The extraction process was repeated on the residue using 50ml alcohol and the final solution was brought to volume with addition of 80% alcohol. The residue was dried and retained for starch analysis.

2.3.2.3. Alcohol Evaporation

20-25ml water was added to the extract solutions to avoid evaporation to dryness. Two methods were used for alcohol evaporation - (a) Extract solution was placed in a quickfit flask which was fitted to a Rotavapor (Buchi Rotavapor 110, Orme Scientific Ltd, Middleton, Manchester, UK) connected to a high vacuum pump and compressor or (b) Extract solution was placed in a water bath at 85 $\pm 2^0$C for one to two hours until all alcohol had evaporated. The evaporated solution was made up to volume with distiled water and heated to $80^0$C to soften gummy precipitates and break up insoluble masses.

2.3.2.4. Clarification of Solution

All clarification was done using lead acetate (AOAC Methods, 1980). Briefly, a saturated solution of neutral lead acetate was prepared, and a few drops added to the

test solution to produce a flocculent precipitate. The precipitate was filtered and the lead acetate taken out of solution by precipitating it with solid anhydrous sodium carbonate ($Na_2CO_3$) and filtered through dry paper. This method proved quite reliable resulting in almost colourless solutions which are essential for the Somogyi-Nelson method.

### 2.3.3. Determinations

Glucose standard solutions, low alkalinity copper reagent and arsenomolybdate colour reagent were all prepared as detailed in Appendix 1.

#### 2.3.3.1. Sugar analysis

(a) Reducing sugars: 1ml test sample or blank (distiled water) was mixed with 1ml copper reagent and placed in vigorously boiling water for 15 minutes. After cooling, 2ml arsenomolybdate was added and the solution diluted to volume (10ml). Absorbency was read in macro-cuvettes at 500 nm using a visible spectrophotometer (Phillips PU 8650, Pye Unicam Ltd, Cambridge, UK).

(b) Non-reducing sugars: This involved inversion of non-reducing to reducing sugars with invertase, determination of total sugars from which the reducing fraction was subtracted to obtain non-reducing sugars. Briefly, 100 units Invertase scales (I-9253, Sigma Chemical Co., St. Louis, MO, USA) was dissolved in 1ml 2M pH 4.5 acetate solution and added to 1ml test sample solution. This was incubated at $55^0$C for 2 hours and analysed for reducing sugars as in (a) above.

(c) Starch: Determination involved hydrolysis of starch with glucoamylase (Thievend et. al., 1972). Briefly, 25ml water was added to sugar free plant residue and heated to $80^0$C for 1 hour. Thereafter, 2.5ml acetate buffer (pH 4.5), 25ml water and 5ml enzyme preparation [50mg Amyloglucosidase (E.C 3.2.1.3., Sigma Chemical Co., St Louis, MO, USA) in 1ml water] were added and the solution heated in a shaking

water bath for 1 hour at $55^0$C. The solution was filtered, diluted to volume and aliquots analysed for reducing sugars as in (a).

CHAPTER 3.

# SEEDLING GROWTH AND DEVELOPMENT.

## 3.1. INTRODUCTION.

Although macadamia has been studied extensively since its development as an important 'nut crop', the early development of the plant has not received much attention. Much research has concentrated on the growth and development of mature trees and fruit growth. This emphasis is the result of the need by growers to relate yield and growth in direct and simple terms. Apparently very little is known about the growth and development of macadamia seedlings and young plants.

Seedling growth studies are important and can be of great use in the understanding of growth and development of crop plants. In tea (Bond, 1942; 1945) studies on vegetative growth have been used to provide an anatomical background for investigations of 'phloem necrosis' disease. Growth studies can also be used to determine the course of dry matter accumulation and even its distribution among different organs in crop plants (Rutter, 1957). There is also the possible importance and relevance of seedling studies being used for understanding growth in shoot systems of mature trees.

Mature macadamia trees are characterised by their growth which is achieved through a cyclic pattern of vegetative flushes (growth flushes) with flushing peaks in summer, spring and autumn depending on several factors including location, temperature, and nutrient availability (Allan, 1983: Stephenson and Cull, 1986b; Stephenson and Gallagher, 1983).

Cyclic growth seems to be a characteristic of most tropical and subtropical tree species including mango (Mangifera indica), cocoa (Theobroma cacao) and cashew (Anacardium occidentale). Shoots do not grow continuously, but pass through

alternate phases of growth and dormancy (Romberger, 1963). The growth period is characterised by the expansion of leaves and elongation of the shoot. During dormancy the shoot length remains constant and no new leaves expand. If the growth flush occurs at regular intervals the resulting growth pattern is called '**rhythmic** or **periodic**', whereas if it is irregular it is called '**intermittent** or **recurrent**'.

Rhythmic growth has been regarded either as a manifestation of endogenous rhythms (Borchert, 1969; Greathouse, Laetsch and Rhumney, 1971), or the result of environmental effects (Alvim, 1964). Proponents of the theory that rhythmic growth is under environmental control suggest that variations in climatic factors such as temperature (Humphries, 1944; Greenwood and Posnette, 1950) and moisture stress (McDonald 1932) in some way regulate the cyclic flushes. Humphries (1944) working on cocoa trees concluded that flushing was largely controlled by temperature and suggested that the weekly mean of the daily maximum temperature must be at least 28.3$^0$C for flushing to be initiated. This was partly supported by Sale (1968) who reported that flushing in young trees of cocoa, grown in controlled environmental rooms at temperatures between 23.3 and 30$^0$C, occurred at all temperatures but was considerably greater at the higher temperature.

The theory that growth flushes are controlled by endogenous mechanisms is ~~also~~ supported by evidence that rhythmic growth persists even under controlled environmental conditions (Sale, 1968). The importance of inhibitor/promoter balance in leaf flushing has been reported in cacao (Alvim, Alvim, Lorenzi and Saunders, 1974). Their results suggested that the termination of a leaf flush was due to the translocation of more abscisic acid (ABA) from leaves to growing points and the diversion of large amounts of cytokinins from roots to leaves. It was suggested that this may lead to a gradual increase of inhibitor/promoter ratio in the shoot tips. This mechanism would indicate that there might be a critical leaf area at which point ABA action totally overcomes cytokinin effects. Hence for growth to be resumed following

this stage of correlative inhibition the critical leaf area would have to be somewhat reduced. The induction of abscission layers would act as barriers against diversion of root cytokinins to leaves and leaf ABA to shoot tips thus triggering growth flushes. Shedding of leaves may also influence the hormonal balance.

For obvious practical reasons, studies on growth of older trees are mainly restricted to measurements of increase in height, girth, and volume of wood, foliage and yield. Briggs, Kidd and West (1920) conceived methods for quantitative analysis of plant growth involving techniques which depend on taking periodic samples of a given population of plants in order to determine rate changes in dry weight and distribution of those changes amongst various plant parts. Such studies have been conducted on several crops including cocoa (Goodall, 1949), pine (Rutter, 1957) and tea (Bond 1942; 1945). However, there are considerable practical difficulties in carrying out measurements of this kind on a large subtropical tree such as macadamia. Although it was possible to carry out some investigations on trees in Malawi as part of this study (Chapter 6), it was necessary to carry out much of the work under UK glasshouse conditions, necessitating the use of small seedlings. As experimental material, seedlings have the advantage of manageable size, permitting replication, and the use of conditions permitting some environmental control.

The danger of drawing conclusions from studies on seedlings about the growth of older trees is fully recognised, but it seems a reasonable working assumption that the information from such studies will provide at least some insight into the growth physiology of the individual. Of course the growth of macadamia seedlings is of intrinsic interest also, as they provide the rootstocks for grafted trees.

Studies on seedling growth were conducted with two main objectives:
(1) To establish the pattern of growth of macadamia seedlings. and (2) To investigate the pattern of dry matter accumulation in various organs during growth.

## 3.2 Materials and Methods

### 3.2.1. Germination

Observational studies were conducted to establish a time course for germination and determine whether germination could be enhanced by mechanical treatments. Treatments included sowing nuts with or without shells. Nuts were cracked using a heavy duty hydraulic press.

### 3.2.2. Quantitative study of seedling growth

This study was conducted in order to determine the pattern of growth in greenhouse grown seedlings from germination. Newly emerged seedlings were initially grown in 0.6 litre plastic pots for 1 month before being repotted into 2 litre pots for the rest of the period. Plants were destructively sampled from April to June 1990. 10 plants of uniform age were used every week for 3 months, beginning 2 weeks from seedling emergence. Due to large variations in the time taken for individual seeds to germinate the date of emergence was taken as a reference point as opposed to sowing date. Roots were washed free of soil as completely as possible. However, many of the finer roots must have been lost.

Roots, stems and leaves were separated. Measurements were carried out on total stem growth, total leaf and root fresh and dry weights, and total leaf area. The material from each individual plant was separately dried at $105^0$C for 24 hours in a forced draught oven.

### 3.2.3. Seedling growth pattern

A study, on the pattern of growth and development of individual macadamia seedlings, was conducted over a five month period from March 1989. This was aimed at developing a quantitative description of cyclic growth in macadamia, determining the differences between individual plants in particular aspects of the growth cycle, and comparing growth under glasshouse and closely controlled environmental

conditions of the growth cabinets. Two groups of 48 plants of uniform emergence were maintained respectively in the glasshouse and growth cabinet under conditions already described in Chapter 2.1. Data were collected non-destructively at weekly intervals for 5 months. The measurements included: increases in extension growth, leaf numbers and area and a subjective assessment devised to monitor developmental stages of the plant.

### 3.2.4. Seedling defoliation and decapitation

Decapitation and defoliation treatments were carried out, aimed at disturbing the balance between shoot growth and foliage area in seedlings. The treatments were carried out on 3 month old seedlings, the experiment laid out in a randomised block design with the 6 treatments replicated 4 times ( single seedling replicates) as follows:

(1) Decapitation of apical bud

(2) Complete defoliation

(3) Defoliation of non-hardened leaves

(4) " of hardened leaves

(5) Decapitation and complete defoliation

(6) Control - no treatments.

Data on extension growth, leaf numbers and leaf area were collected after 3, 6 and 12 weeks. Data in these series of experiments were subjected to ANOVA analysis in Minitab.

## 3.3 RESULTS

### 3.3.1. Seedling germination

In general, the first batch of seeds had very low viability, an average of 24.6%. Germination commenced 4 weeks after sowing. Shells on intact nuts opened up after 3 weeks exposing the developing embryo. The radicle emerged first with the plumule appearing 3-5 days later. The plumule elongated rapidly producing leaves in quick succession. Germination was higher in intact nuts than in partly cracked nuts, with levels of 40% and 27% respectively after 5 weeks. Fully exposed kernels decomposed within a week.

### 3.3.2. Quantitative study of seedling growth

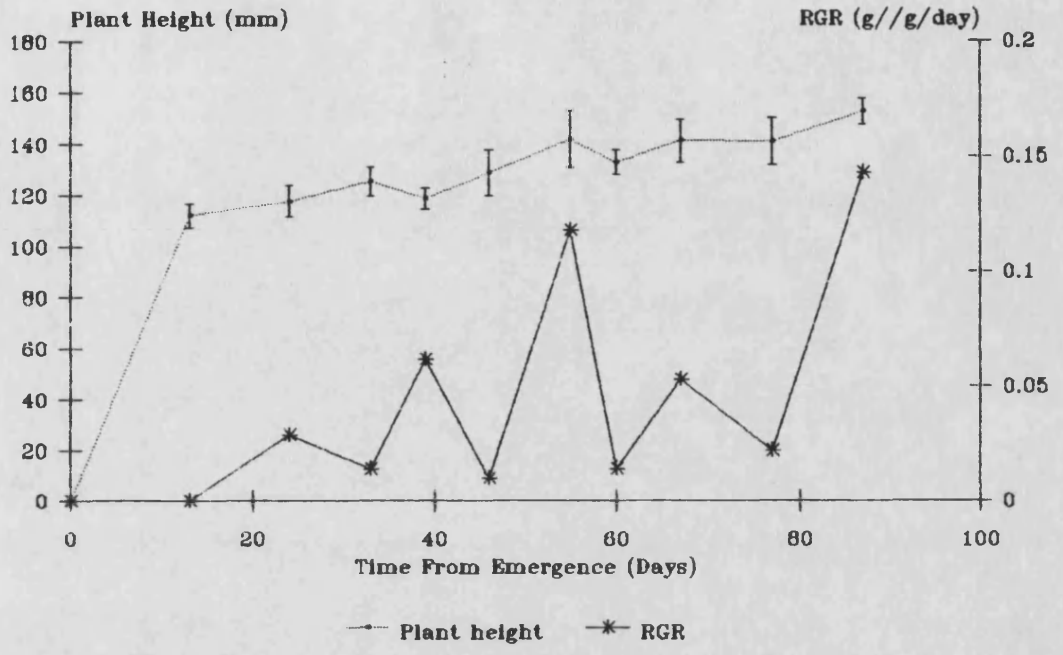
Following seedling emergence there was a period of very rapid extension growth in the first two weeks (Fig. 3.1) followed by a rather steady growth pattern for the next 11 weeks. In fact, on average 73% of the total extension growth over the 13 week period occurred in the first 2 weeks. Fig. 3.1 also includes data on relative growth rates (RGR or *r*) for each period between two successive samples calculated by the formula (Rutter, 1957):

$$r = \frac{\log_e W2 - \log_e W1}{T1 - T2}$$

where W1 and W2 are plant dry weights at times T1 and T2.

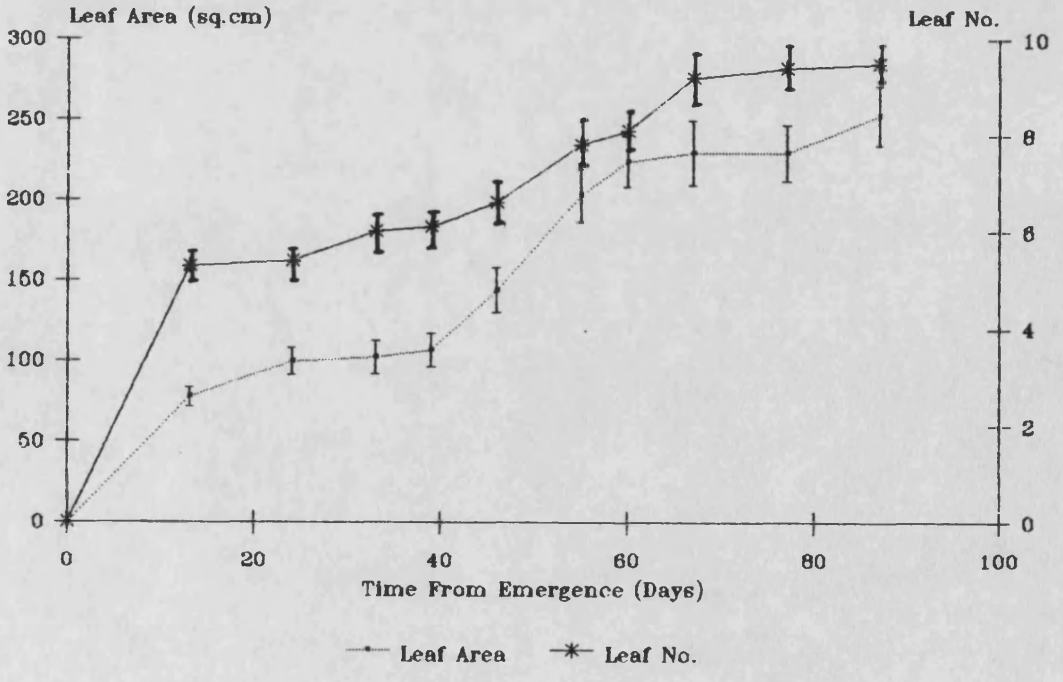
The formation and development of leaves shows some periodicity (Fig. 3.2). The first two to four leaves were formed following the initial stem extension growth in the first two weeks. No more leaves were formed until after six weeks. This periodic formation of leaves is repeated every four to six weeks. Leaf development shows a similar pattern with rapid expansion of the first set of leaves followed by a period of steady increase in leaf area until after seven weeks when there is more leaf area increase following development of another set of leaves.

Fig. 3.1 Stem Extension Growth and Relative Growth Rate (RGR) of seedlings over a period of time.




Vertical lines represent ±SE (n=10)
RGR = Relative Growth Rate (g/g/day)

Fig. 3.2 Increases in total leaf area and leaf numbers in seedlings over a three month period.




Vertical lines are ±SE (n=10)

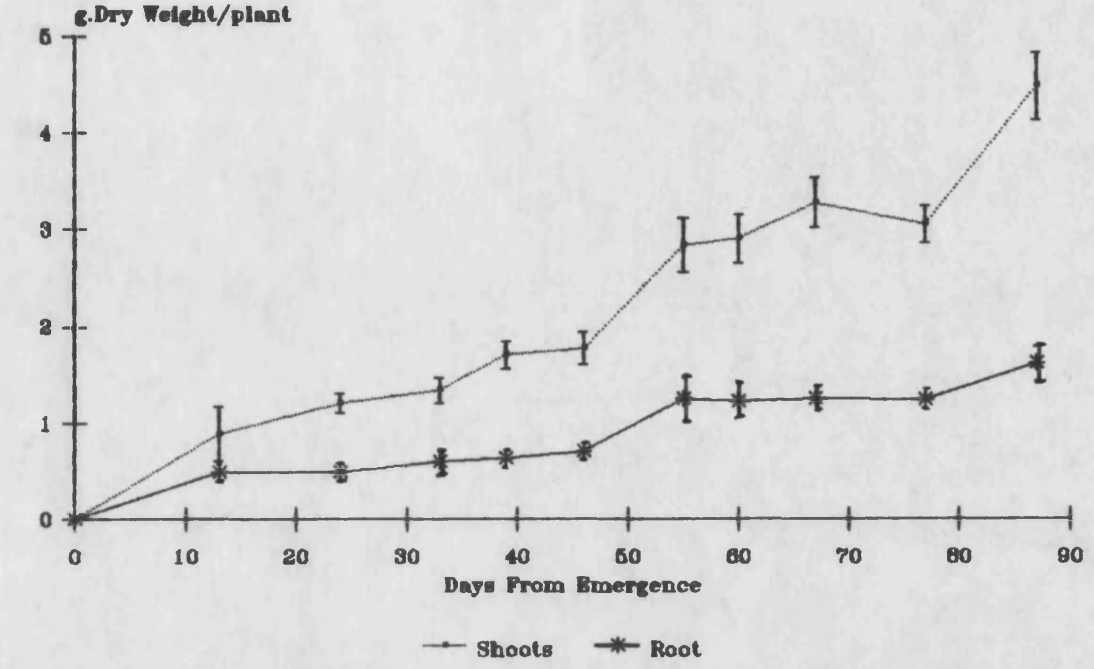
The accumulation of dry matter in the shoots and roots (Fig. 3.3) followed the same trend as extension growth and leaf development. It should be noted that cotyledons were excluded from all dry matter determinations. There was a steady accumulation of dry matter in both shoots and roots during the first six weeks, followed by a rapid increase soon after and another one after thirteen weeks. More dry matter accumulated in the shoots than did in the roots at all stages of development. The differential was rather small in the first six weeks but increased afterwards. The shoot:root ratios (Fig. 3.4) show an interesting pattern. Following emergence there was rapid increase in the ratio for three weeks, followed by periodic fluctuations in the ratio as if the plant was trying to maintain a balanced ratio between shoots and roots. Although the differential between root and shoot dry matter steadily increased after six weeks, the shoot;root ratio was relatively stable at between 2.5 and 3.

### 3.3.3. **Seedling growth patterns**

#### 3.3.3.1. Growth phases

A close examination of individual plants during growth showed some of the related morphological developments. Plants underwent a '**resting**' phase which was marked by an apparent lack of visible activity of the apical bud and cessation of extension growth and little leaf growth (Plate 3.1). Apical buds on such plants remained closed, thin and flat. They either turned dark or remained green. Seedlings remained in this phase for up to two weeks. This was followed by a '**growth**' phase which was initiated by swelling of the apical buds. The bud scales became free at the tip and along the margins so the bud appeared to be breaking (Plate 3.2). Extension growth then commenced, followed by development of leaf initials from which a few successive leaves (4 to 6) developed. The 'growth' phase, or more appropriately 'growth flush', was marked by rapid extension growth and leaf area increases. Hence the growth flush involved both stem extension growth as well as leaf development.

**Fig. 3.3 Pattern of shoot and root dry matter accumulation in seedlings grown for three months**




Vertical lines are ±SE (n=10)

**Fig. 3.4 Changes in total plant dry wt. and shoot:root ratios over a three month growing period in macadamia seedlings.**

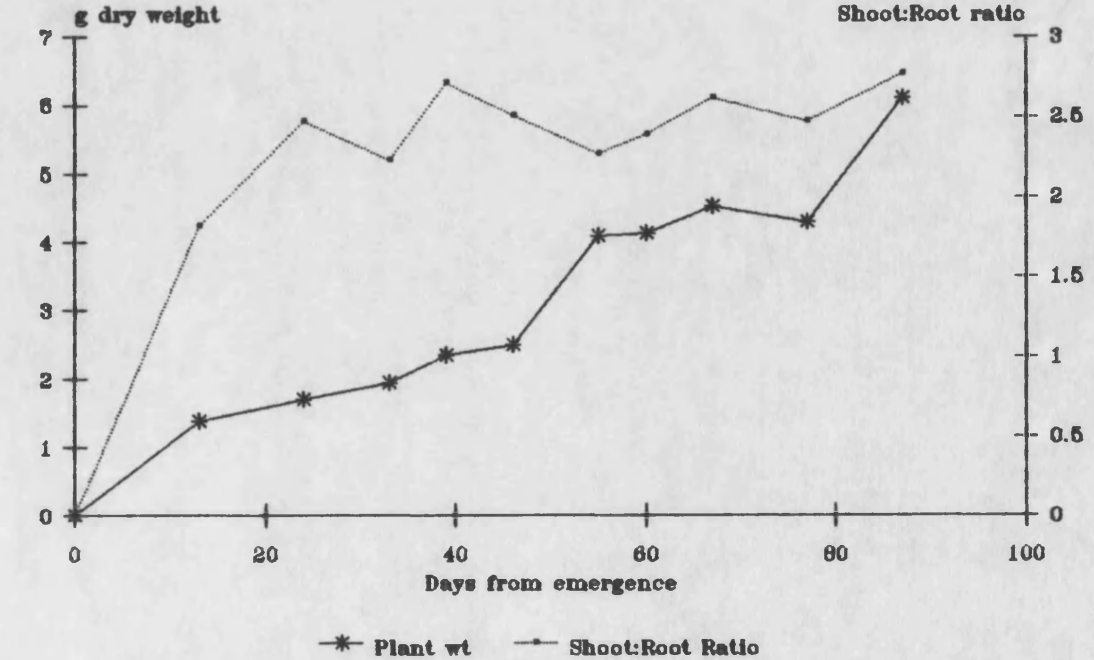


Plate 3.1 Day 0. Apical bud of macadamia seedling in a 'resting phase'. Note that the bud is closed and flat, all leaves are hardened and there is no stem extension.

Plate 3.2 Day 4. Growth initiation in seedlings. Apical bud is breaking into the first pair of leaves followed by initiation of stem extension growth.

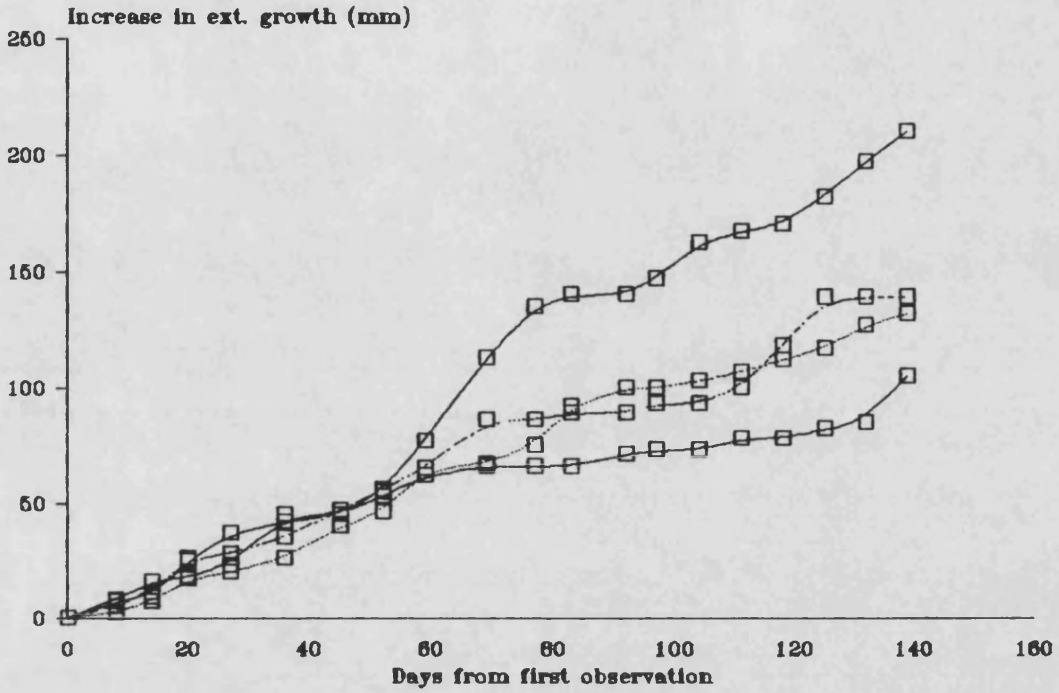
3.3.3.2. Extension growth

Extension growth for up to 21 weeks showed a continuation of earlier trends. Since plants older than 13 weeks were not subjected to destructive assessments, data on dry matter distribution during these later stages is not available. Increases in extension growth both in the glasshouse (Fig. 3.5a) and growth cabinet (Fig. 3.5b) followed patterns of rapid increases in growth followed by those of steady increases or resting. This is more apparent in cases where growth on individual plants was followed for the entire period of 21 weeks. There were variations between plants in total extension growth as well as in the periodicity of the phases of growth. Hence stem elongation occurred at different times in each seedling and was not of constant duration between plants nor indeed within any individual plant.

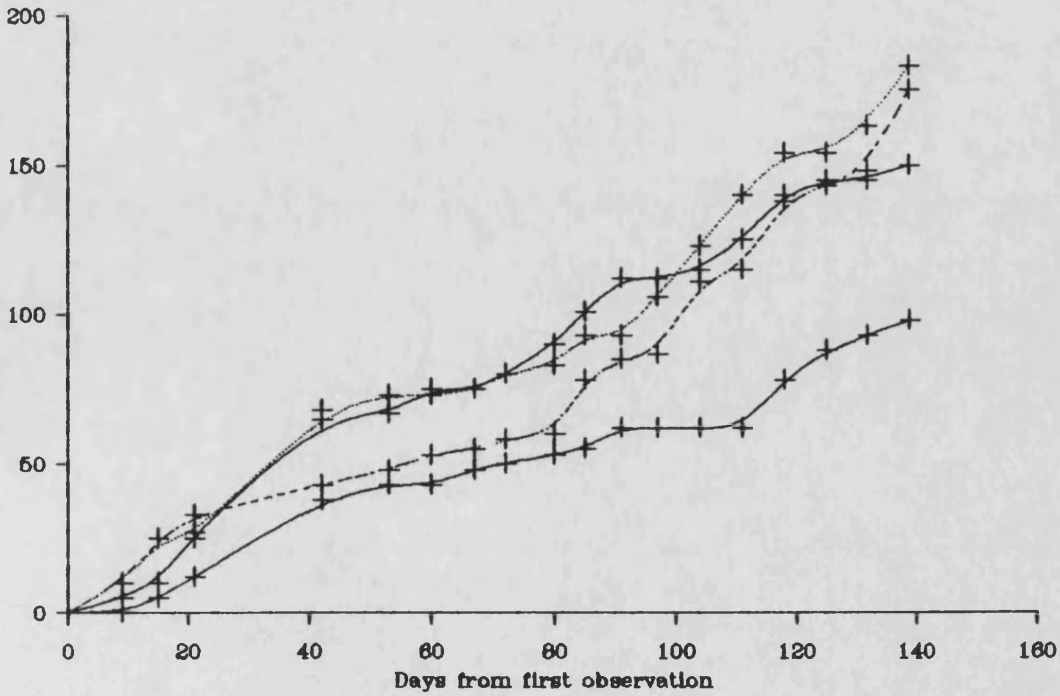
3.3.3.3. Leaf development

Leaf growth was initiated by the presence of leaf initials, normally 2 to 3 per node, which were held together before separating to form distinct leaves. Separation commenced when initials were 5 to 10mm long and completed when they were 15 to 30mm (Plates 3.3 and 3.4). Newly separated leaves were still folded over the midrib (Plate 3.3). They then unfolded, expanding rapidly and stretching outwards. Leaves were fully unfolded in about two weeks from initiation (Plate 3.4). Leaf unfolding inevitably involves expansion. In effect the process of expansion is continuous, but two stages could be distinguished by a change from the rolled form into a flat leaf. Leaf expansion occurred in two phases. Firstly, the leaf increased in width which was followed by an increase in length before attaining full size. At full expansion leaves were up to 280mm long and 40mm wide and full size was attained within 3 weeks from unfolding. The final stage of leaf development is involved lignification. Leaves slowly attained a brittle nature, became dark green and developed sharp spines or serrations along the edges.

**Fig. 3.5 Increases in extension growth of plants grown in (a) Glasshouse, and (b) Growth cabinet for 5 months.**




(a) Glasshouse




(b) Growth cabinet. Each line represents 1 plant and observations commenced 14 days from emergence.

Plate 3.3 Day 11. Flush leaves separated but still folded over the midrib. A second pair of leaf initials is visible and stem extension growth is occurring.

Plate 3.4 Day 18. Flush leaves fully unfolded and expanding. The second pair of leaves is separated but still folded over the midrib.

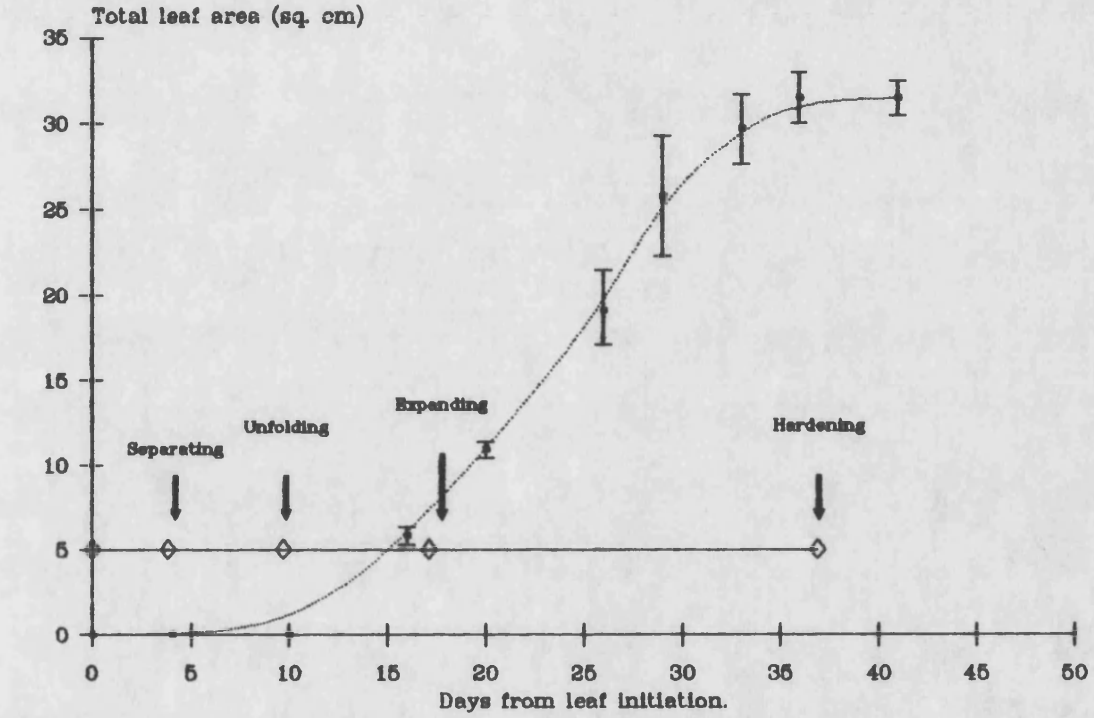
The time sequence of individual leaf development has been summarised in Fig. 3.6. Leaf area data was averaged from a pair of leaves produced on the same node from 8 individual seedlings of the same age. The data show the increases in leaf area from initiation to complete lignification. It is clear that, on average, leaf expansion commenced two weeks after leaf initiation, and reached its maximum rate a week later and ceased by the fifth week. The leaf expansion phase was indicated by the very high rate of leaf area increase for about two weeks which was then followed by no further increase during hardening.

3.3.3.4. Growth flushes

There were great variations in growth flushes between plants and even between successive flushes in a single plant. Two types of flushes were noted; short duration and long duration flushes (Table 3.1.). Overall, of the 13 flushes observed in 5 plants, 11 were of short duration lasting about 3 weeks, while only two were of long duration lasting about 9 weeks. Obviously the longer duration or 'major" flushes resulted in more extension growth and the development of a significantly larger number of leaves and longer internodes. Figs. 3.7 and 3.8 show leaf area increases and patterns of leaf development on short and long duration flushes respectively. Leaf areas were measured non destructively on developing leaves from initiation to complete lignification.

There is a clear variation in patterns between the two types of flushes. In the short duration flush, several leaves on successive nodes in a single flush developed simultaneously within a short time and hardened followed by a short intervening 'rest' period before the next set of leaves were initiated or before next flush (Fig. 3.7). In the major flushes there was continuous initiation and development of leaves on successive nodes but in a sequential manner (Fig. 3.8). A set of leaves was initiated and began expanding before the next set in the same flush cycle was initiated. Each

Fig. 3.6 Course of Leaf Development of a single leaf on a macadamia seedling




Vertical lines represent ±SE (n=16)

Table 3.1 Growth pattern of short duration and long duration shoot flushes on 5 seedlings grown under glasshouse conditions.

| Parameter | Shoot Flush Type | | | |
|---|---|---|---|---|
| | Short duration | | Long duration | |
| | Mean | SE(±) | Mean | SE(±) |
| Flush duration (days) | 23.9 | 2.2 | 66.0 | 3.0 |
| Rest period (days) | 11.7 | 1.1 | 6.0 | 0.5 |
| Total shoot length (mm) | 32.4 | 4.3 | 106.5 | 4.4 |
| Stem growth rate (mm/day) | 1.55 | 0.21 | 1.62 | 0.44 |
| Number of internodes | 2.1 | 0.21 | 4.5 | 0.5 |
| Length of internode (mm) | 16.1 | 2.0 | 23.4 | 2.9 |

Leaf area (sq.cm)

Fig. 3.7 Pattern of leaf development in two successive minor flushes. Similar line styles denote leaves on same node.

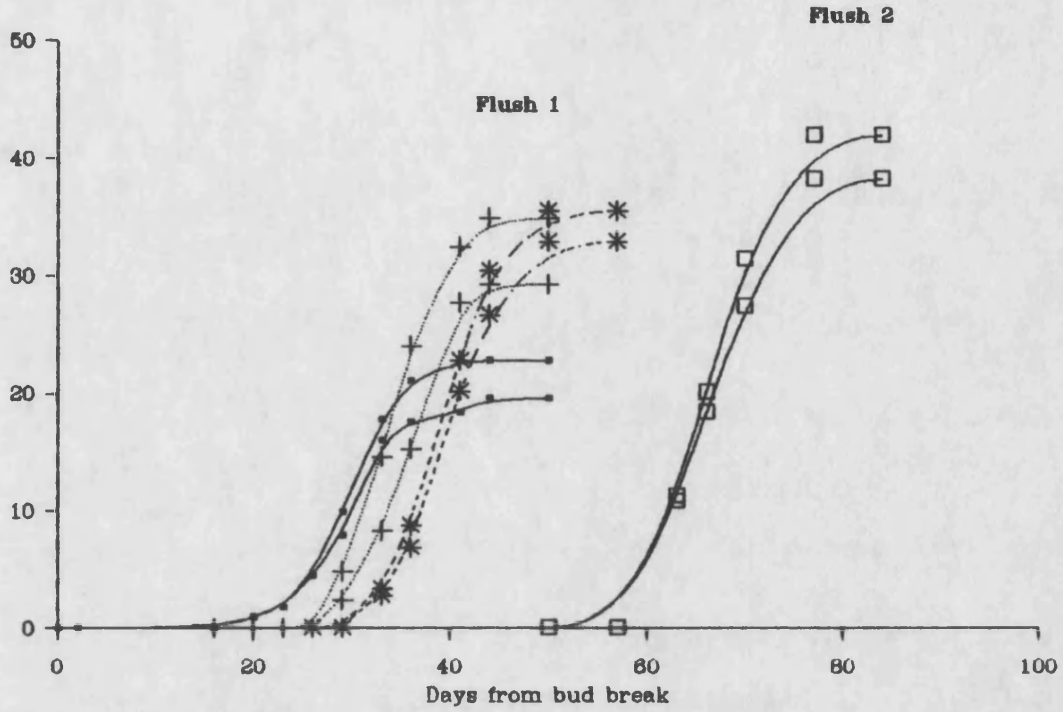
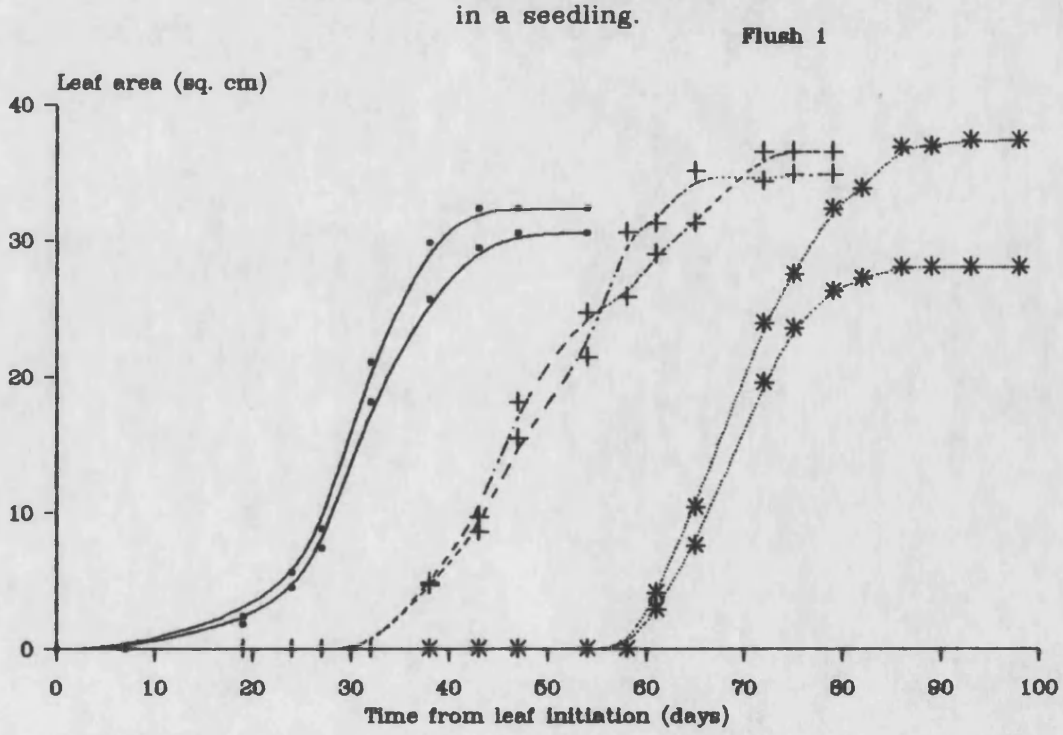



Fig. 3.8 Pattern of leaf development during the course of one major flush in a seedling.




Similar line styles denote leaves on same node.

– Trend used

cycle would produce up to 12 leaves. There were no differences in individual leaf areas between the two flush types

### 3.3.4. Defoliation and decapitation

Table 3.2 shows changes in stem elongation and leaf area following decapitation, and defoliation of hardened and non hardened leaves. Complete defoliation and decapitation resulted in immediate death of the plants. Significant differences in extension growth were obtained between various treatments after 3 weeks (P=0.01) and 6 weeks (P=0.05) (Appendix 2A). After 3 weeks, plants whose non-hardened leaves had been removed elongated the most, whereas decapitated plants had the least growth. Decapitated plants subsequently recovered following release of axillary buds and by the end of 12 weeks had the most growth. This gives an indication of the strength of apical dominance in macadamias. All treatments resulted in more extension growth than the control showing that there was a stimulation of growth following defoliation or decapitation.

The leaf development pattern varied according to treatment. Following the release of axillary shoots in decapitated plants there was a rapid increase in leaf area after 6 weeks due to expansion of the numerous leaves. On plants from which non-hardened leaves had been removed, the response was by rapid extension growth and expansion of new leaves. At the end of 12 weeks all treated plants had higher leaf area than controls also showing stimulation of leaf area growth following defoliation and decapitation.

Table 3.2 Effects of stem decapitation and leaf defoliation on increases in stem elongation (mm) and leaf area (sq. cm).

| | Time after treatment (weeks) | TREATMENTS | | | | | |
|---|---|---|---|---|---|---|---|
| | | Control | Decapitated | Defoliation (Exp. leaves) | Defoliation (Hard leaves) | SED | LSD |
| Increase in stem length (mm) | 3 | $16.5^{a}$ | $1.2^{a}$ | $37.5^{b}$ | $14.0^{a}$ | 7.21 | 16.08 |
| | 6 | $39.2^{ab}$ | $28.7^{a}$ | $56.5^{b}$ | $21.0^{a}$ | 9.59 | 21.39 |
| | 12 | $51{\cdot}0^{a}$ | 66.0 | 61.0 | 66.0 | 9.51 | NS |
| Increase in leaf area (sq. cm) | 3 | 56.5 | 40.7 | 27.7 | 89.5 | 25.16 | NS |
| | 6 | 125.5 | 84.2 | 141 | 159.0 | 29.44 | NS |
| | 12 | 157.5 | 248.5 | 193.8 | 220.5 | 48.22 | NS |
| Rate of stem elongation (mm/day) | 3 | $0.82^{a}$ | $0.06^{a}$ | $1.8^{b}$ | $0.7^{a}$ | | |
| | 6 | 1.14 | 1.37 | 0.95 | 0.35 | | |
| | 12 | $0.29^{a}$ | $0.34^{a}$ | $0.12^{a}$ | $1.12^{b}$ | | |
| Rate of leaf area increase ($cm^2$/day) | 3 | $2.82^{b}$ | $2.03^{b}$ | $1.38^{a}$ | $4.47^{c}$ | | |
| | 6 | 3.45 | 2.17 | 5.66 | $3.47^{a}$ | | |
| | 12 | $0.80^{a}$ | $4.10^{b}$ | $1.31^{a}$ | $1.53^{a}$ | | |

Values with different letters within each row are significantly different at P=0.05.

## 3.4. Discussion

With oil levels of over 75% in the cotyledons, macadamia seeds seem to have adequate reserves for germination and initial growth. Initially the seeds seem to require just enough moisture for the embryos to germinate, hence pre-cracking the seeds leads to excessive moisture levels and to seed decomposition. The low viability obtained was most likely as a result of seed ageing. Hamilton (1957) reported that macadamia seeds stored for long periods showed progressively less germination and failed to germinate after 12 months. The period was even less for nuts which were loose in the shell. The initial seeds used here in October were harvested in the previous March. Although viability was not completely lost, it was rather low. Pre-soaking softens up the shell and enables it to be saturated with moisture which is made available to the developing embryo. The seeds are not in direct contact with this moisture. As a result growth is hastened. However, pre-soaking of seeds with cracked shells just hastens their decomposition.

Most of the dry matter accumulation in the shoots of seedlings should be attributed to leaf growth as stem growth was a relatively minor component of the seedling weight. Leaf growth in the early stages was continuous in that at all times leaves were either being initiated or expanding even when stem extension had ceased. Hence for the 13 week period there was no point at which the shoot was devoid of some growth. There was an apparent tendency for the plants to maintain some balance between root and shoot growth. Increases in shoot dry matter were accompanied by increases in the roots, but the shoot dry matter was always consistently higher, approximately two and half times that of the roots. This prompts the suggestion that either the macadamia has a poor root system or that the roots are efficient that only small root volumes are required to support large shoots.

The shoot:root ratios in macadamia are similar to those reported in cocoa by Goodall (1949). However, they differ greatly to those reported in Carya illinoensis (pecan)

where seedling root dry weight was twice that of shoot dry weight (Wood 1984). In macadamia seedlings, shoot and root growth seem to be adjusted and correlated over a period of time to maintain a balance. It has been noted that plants normally maintain a rather constant ratio between roots and shoots (Wareing, 1970). There is, therefore, a tendency to restore any alterations in the ratio by compensatory growth of the organs. This reflects a basic biological principle that any multicellular organism can survive only if it maintains a functional balance between its various parts. It is not clear whether the relationship between shoot and root growth in macadamia is one of phased growth or that one process occurs at the expense of another.

The growth flushes or phases seem to be continuous in the very young seedlings. This confirms earlier findings on macadamia seedlings by Cormack and Bate (1976) Shoot flush growth has been reported in cocoa (Goodall, 1949), coffee (Alvim, 1977), and tea (Bond, 1945). However, these reports give no clear indication of root development patterns during shoot flushes. In tea, flushing is considered to be a function of apical activity and primordial growth. Like cocoa (Sale, 1968), but unlike tea (Bond, 1945) and C. viminalis (Purohit and Nanda, 1968) leaf initiation in macadamias seems rhythmic and in phase with shoot elongation and leaf expansion. However, there seems to be a lot of variation between individual plants, with each plant showing independent rhythmic growth. The major or long duration flushes, which occurred rather infrequently, would seem to be a modification of the normal flush behaviour in seedlings. The strong leader shoots showed aperiodic growth producing up to eight or more leaves with normally elongated internodes or very shortened ones without any intervening dormancy periods.

The apparent causes or stimulants of growth flushes in macadamia cannot be adequately explained. There are two possible explanations: The flushes could have been triggered endogenously through hormonal control or could involve a response to some environmental factors. Since plants were grown under controlled environmental

conditions, the effects of temperature, humidity, fertiliser and water as reported by Humphries (1944), Greenwood and Posnette (1950) and McDonald (1932) could not be held directly responsible for triggering growth flushes in the seedlings. The persistence of rhythms, the asynchronous growth and the obvious lack of correlation between the rhythms and environmental conditions suggests a strong influence of endogeneity in macadamia seedling flushes.

It is possible that endogeneity could be influenced by some environmental factor such as changes in water balance within the plant. Greathouse et. al., (1971) suggested that a disturbed water balance at the end of each growth flush may be responsible for the arrest of shoot growth. Since new leaves at the beginning of each flush expand rapidly, the transpiring leaf surface is likely to increase much faster than the capacity of the poorly developed roots for water absorption or possibly the transport capacity of the xylem vessels in the elongating shoot. As transpiration exceeds the amount of water supplied to the shoots, a water deficit would originate in the shoot which would inhibit cell enlargement both in the subapical meristem and expanding immature leaves, thus arresting shoot and leaf growth simultaneously. The high shoot:root ratio shown here fits in well with this argument. It could also fit the endogeneity theory where flush growth is a consequence of the balance between ABA in the roots and cytokinins in the roots. The poor root system in macadamia may not be able to produce enough cytokinins to offset the balance of ABA produced in the heavy foliage. This may lead to inhibition of terminal growth until such a time that the balance is offset by cytokinins following more root growth.

In a quest to understand endogenous rhythms in young plants, Borchert (1973) developed a model based on the assumption that endogenous rhythms can result from feedback interaction between two potentially continuous growth processes like shoot and root growth if the slower process was rate-limiting for the faster one. Hence rhythmic growth in trees would be a consequence of feedback mechanisms needed for

maintaining a constant shoot:root ratio. This model was based on environmental influences on rhythmicity. In cases where such influences are controlled then perhaps the stimulus for rhythmic behaviour would come from changes in hormone balance resulting from changes in the shoot:root ratios.

Clearly, if the shoot growth rate is significantly higher over a long period of time, as it was here, temporary arrest of shoot growth becomes necessary to maintain balanced growth. If this is so, then the flush cycle in plants such as macadamia, rubber and cocoa which have high shoot:root ratios could be considered to be a mechanism for balancing growth by arresting stem elongation, whilst allowing for root development. Such temporary arrest of shoot growth may depend on deficiencies in any of the various substances which roots supply to shoots; water, mineral salts, hormones or other. In line with this, it is possible that the flushing pattern could be as a result of exogenous hydroperiodic stimulus leading to changes in internal moisture balances which affect abscisic acid and cytokinin balances (Wareing, 1978). This would encompass effects of environmental effects, in this case moisture, and endogenous hormonal effects. In the macadamia the root seems to have limited growth and may, therefore, play an important role in flushing through limitations in moisture uptake to maintain the relatively large shoots.

# CHAPTER 4.

# SEEDLING GROWTH AND ASSIMILATE PARTITIONING.

## 4.1. INTRODUCTION

The increased productivity of crop plants over that of their wild ancestors is due primarily to improved distribution of dry matter to the harvested part of the plant, rather than to increased production of total dry matter (Gifford and Evans, 1981). Hence a better understanding of the partitioning of assimilates with plant growth is of paramount importance for the further development of crop yields.

Carbohydrates are a major reserve of woody plants (Priestley, 1962; Cheffins and Howard, 1982). The insoluble carbohydrate most commonly found in trees, both above and below ground, is starch (Tromp, 1983); the soluble carbohydrates vary greatly between species. Carbohydrates serve as sources of energy (sugars) and stores of energy (starch). They also form a major portion of the supporting tissue of plants (cellulose). Carbohydrates, in one form or another, are essential for the maintenance and development of new meristems; (i) as a source of respirable substrate which can provide the energy for synthesis and growth, (ii) as the basic components of structural polysaccharides and their derivatives which are incorporated in new cell wall material, and (iii) as molecules that can be broken down to forms which, after the incorporation of such chemical groupings as the amino group, can be synthesized to provide components for the cytoplasm. Starches, formed by the conjugation of glucose units, are the most important glucosans in woody perennials. They are deposited in the cells of dicotyledons whenever a high level of soluble sugars is attained and are reconverted to soluble sugars during times of sugar deficiency and in cold weather. Conversions of starch to sugars also occur at times of most active growth when reserves are used in support of meristematic activity in shoot apices, cambial cells and young fruits.

The mobilisation and utilisation of assimilates in crop plants has received much attention. Depletion of starches during shoot growth in macadamia (Cormack and Bate, 1976) and avocado (Scholefield, Sedgley and Alexander, 1985) suggests that growth may depend, partly, on stored carbohydrates. Wormer and Egabole (1965) have shown that coffee continues to develop even after the starch content in the wood is totally depleted and suggest that the crop might first withdraw starch from wood and only then from leaves. However, Patel (1970), found that withdrawal of starch by the developing coffee crop took place from both kinds of tissue concurrently. Stephenson, Gallagher and Rasmussen (1989), working with macadamia bark tissue, argued that the decline in carbohydrate reserves in spring and summer was due to demand by the developing crop rather than of the spring vegetative flush.

Many observations on the distribution of assimilates are consistent with the hypothesis that assimilates are partitioned within the plant in response to demand by the various sinks, arising from either the utilisation of assimilates in growth or their accumulation in the form of immobile food reserves such as starch, lipids and protein (Kriedemann, Loveys, Possingham and Saloh, 1976; Patrick, 1976). The strength of any given sink is measured by its absolute size and its activity (Wareing 1978) i.e Sink strength = Sink size X Sink activity, where sink activity is the potential rate of metabolic uptake per unit weight per unit time.

The supply of assimilates to a sink involves their transfer from the phloem to the sink tissue and this appears to occur via the apoplast so it will involve transfer across two membranes (Troughton 1976); (1) transfer across the plasmalemma of the sieve tube into the apoplast (unloading) and (2) uptake from the apoplast into the symplast of the sink tissue, the rate of which will be measured by its sink activity. The rate at which assimilates are accumulated by a sink can presumably be limited either by the rate of unloading or rate of uptake from apoplast. Research (Reviewed by Patrick and Wareing, 1980) has indicated that some hormones act by stimulating the active

transfer of sugars from the phloem to the surrounding ground tissue, that is by stimulating the unloading process from the sieve tubes into the apoplast and/or active uptake by the ground tissue from the apoplast.

Research in vines has shown the changes in direction of photosynthate movement from a given source leaf (Quinlan and Weaver 1970). From a newly exporting leaf, movement is acropetal whereas with continuing growth of the shoot and production of new leaves movement from the same leaf becomes basipetal. Such movements have been reported in crops such as apples (Priestley, 1962) where leaves on the upper part of the shoot are reported to export assimilates to the shoot apex and young leaves, while lower leaves mainly supply the root system.

Many factors, both intrinsic and extrinsic, act to determine whole plant photosynthesis and, therefore, dry matter accumulation. Paramount amongst the intrinsic factors is the amount of dry matter reinvested in the growth of photosynthesizing tissues, mostly leaves (Milthorpe and Moorby 1979). Sucrose and possibly other phloem-mobile assimilates are loaded into sieve tubes from the apoplast against a steep concentration gradient by an energy-dependent, carrier-mediated transfer process (Geiger, 1975).

Although growth requires a large amount of available carbohydrates, several lines of evidence indicate that cessation of growth of woody plants is seldom due primarily to carbohydrate deficiency. For example, when the annual shoot growth of perennial plants slows down, a substantial carbohydrate reserve is usually still present. In apples, only about one third of the extractable carbohydrate supply was depleted during apple tree growth (Priestley, 1962). Hence carbohydrate supplies in plants often appear to be adequate but growth is inhibited by inadequate transport of substrates to growth centres and by internal blocks to sites of utilisation and conversion into new tissues. Scholefield et. al. (1985) noted that in avocado (Persea

americana) there was a cycling of carbohydrates with maximum levels occurring in early spring, declining during shoot growth to a minimum in autumn followed by an increase at about the time of cessation of vegetative activity, sustained over winter till reaching maximum levels in spring. A clear understanding of this phenomenon in individual crops is essential as it helps in determining timing and application of critical cultural practices such as nutrition, irrigation and general crop management.

### 4.1.1. Aim of study

This study was designed to examine levels of soluble sugar and starch distribution in new leaves, mature leaves and roots of macadamia seedlings and relate them to the growth phases already established in Chapter 3.

## 4.2 Materials and Methods.

Two studies were conducted on 3 and 5 month old seedlings grown in 2 litre pots in the glasshouse under conditions described in Chapter 2.1.

**Study 1.** Three-month old seedlings of M. integrifolia were classified into three groups comprising three plants each. Classification was based on the growth phase of individual plants in September 1990 as being *Dormant, Initiating flush,* and *Flushing.* Plant part samples (leaves and roots) were oven-dried at $95^0$ for one hour and $70^0$C for 4 hours in a forced draught oven prior to being subjected to carbohydrate analysis as described in Chapter 2.3. Total Non-structural Carbohydrates (TNSC) were determined by adding the reducing, non-reducing and starch components expressed as mg. glucose equivalents per g. dry matter

Group 1. Dormant Plants; Seedlings on which extension growth had ceased but still had some expanding leaves. Samples from such plants comprised of;

(i) Topmost leaves which were still expanding.

(ii) Lower leaves, a sample from all mature lower leaves.

(iii) Roots.

Group 2. Initiating Flush; Seedlings on which all leaves were fully expanded and lignified but new leaves were initiating following a bud break. Samples consisted of;

(i) Topmost leaves which were hardened.

(ii) Mature lower leaves.

(iii) Roots.

Group 3. Flushing Plants. Seedlings on which a new flush was well under way. Samples included;

(i) Flush leaves which had just started
expanding.
(ii) Mature lower leaves.
(iii) Roots.

**Study 2.** This was a slight modification of the above and was conducted in September 1991 on 5-months old seedlings. Plants were classified into similar groupings but denoting a more progressive sequence of the growth phases;

Group 1. Dormant Plants. These were in a true sense of visual dormancy. All leaves had hardened and extension growth had ceased. Samples comprised of;

(i) Topmost leaves which were fully hardened.
(ii) Bottom leaves, the lowest pair on the
plant.
(iii) Roots.

Group 2. Initiating Flush. These were plants which had just passed through their dormancy phase and undergoing bud break as indicated by apical bud expansion. Samples consisted of;

(i) Topmost leaves which were hard.
(ii) Bottom leaves.
(iii) Roots.

Group 3. Flushing Plants. This included plants which had a growth flush. Samples comprised of;

(i) Flush leaves, which were expanding.
(ii) Top leaves which were hardened.
(iii) Bottom leaves.

(iv) Roots.

The term *dormancy* is loosely used here to describe the visible plant status and does not imply the general physiological or metabolic status of the plant. In fact the growth phases were established purely on a visual basis.

## 4.3. RESULTS

There were no significant differences in total carbohydrate concentration between growth phases in 3 month old plants (younger plants) (Appendix 2B). Trends, however, indicate that flushing plants had sugar levels 6% and 9% higher than dormant and bud break seedlings respectively (Table 4.1 and Fig. 4.1). Differences were more pronounced in 5 months old plants (older plants) where flushing plants had sugar levels 11% and 35% higher than dormant and bud break seedlings respectively (Table 4.2).

Generally, carbohydrate concentrations were highest in flushing plants and lowest in plants initiating flush or at bud break (Tables 4.1-4.2). Reducing sugars were lowest in both 3 and 5-month old plants at bud break, and similar in dormant and flushing plants (Figs. 4.2a and 4.2b). However, non-reducing sugars were highest at bud break. Starch levels varied slightly with age. In the younger plants they were highest in flushing plants and lowest in dormant plants while in the older seedlings starch was highest in flushing plants but lowest in plants at bud break. In general, older plants had more total carbohydrate concentration than younger ones. This was due to the much higher starch and relatively high levels of non-reducing sugars in the older plants. On the other hand the younger pants had higher reducing sugar levels.

In terms of soluble sugar and starch concentration in the various plant parts there were some interesting trends. In three-month old seedlings the reducing sugar and starch levels were much higher in flush leaves than in lower leaves and roots (Table 4.1 and Fig. 4.3a). Non reducing sugars were highest in roots, and both roots and lower leaves had comparable starch levels. In general, flush leaves had 20% and 55% more total carbohydrates than lower leaves and roots respectively. In five-month old plants (Table 4.2 and Fig. 4.3b) flush leaves had the highest soluble sugar and starch concentrations followed by bottom leaves. Roots had the least levels except for non-reducing sugars. The top hardened leaves had sugar levels lower than those of bottom

Table 4.1. Sugar and starch levels in leaves and roots of 3 month old seedlings at various growth phases. Values are expresed as mg. Glucose equivalents per g. dry matter.

| **Growth Phase** | **Plant Part** | | | | | | |
|---|---|---|---|---|---|---|---|
| | Top leaves | Lower Leaves | Roots | Mean | Sign. | S.E.D | L.S.D (5%) |
| (a) Reducing Sugars | | | | | | | |
| Dormant | 80.11 | 68.98 | 35.74 | 61.61 | | | |
| Bud Break | 59.33 | 65.11 | 25.09 | 49.84 | | | |
| Flushing | 72.89 | 64.1 | 41.36 | 59.45 | | | |
| Mean | 70.77$^{b}$ | 68.5$^{b}$ | 34.06$^{a}$ | | *** | 6.43 | 15.76 |
| Sign. | | | | NS | | | |
| S.E.D | | | | 16.77 | | | |
| (b) Non-reducing Sugars | | | | | | | |
| Dormant | 10.28 | 5.55 | 13.8 | 9.88 | | | |
| Bud Break | 9.8 | 12.96 | 13.77 | 12.18 | | | |
| Flushing | 12.98 | 8.49 | 10.26 | 10.57 | | | |
| Mean | 10.04 | 9.00 | 12.61 | | NS | 2.16 | |
| Sign. | | | | NS | | | |
| S.E.D | | | | 2.43 | | | |
| (c) Starch | | | | | | | |
| Dormant | 76.56 | 31.56 | 62.77 | 56.96 | | | |
| Bud Break | 78.62 | 61.77 | 47.29 | 62.56 | | | |
| Flushing | 72.32 | 73 | 53.87 | 66.39 | | | |
| Mean | 75.83 | 55.44 | 54.64 | | NS | 10.86 | |
| Sign | | | | NS | | | |
| S.E.D | | | | 14.09 | | | |

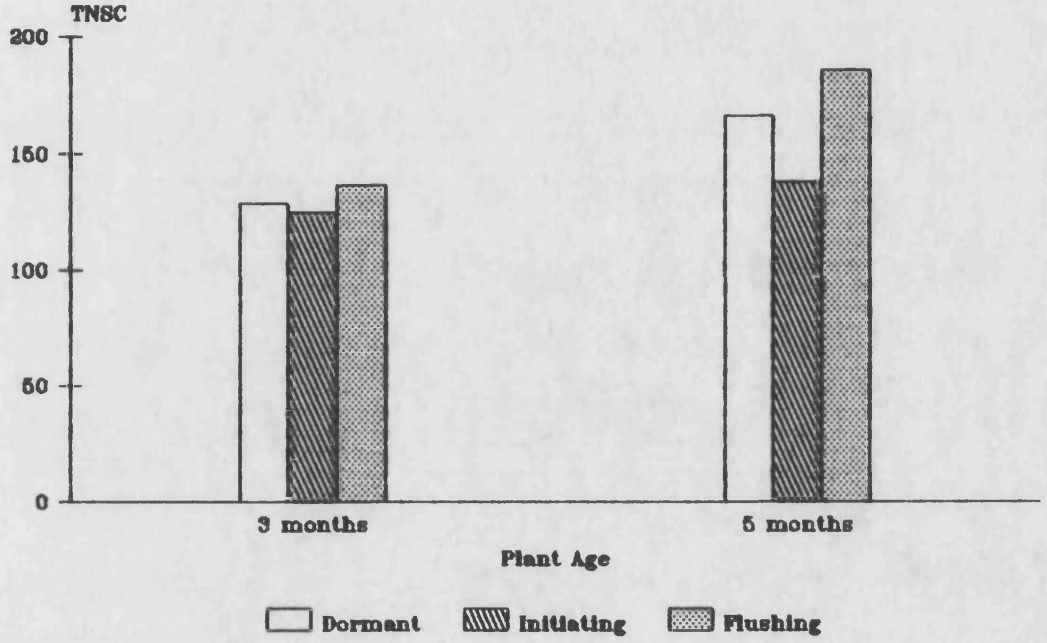
Means in the same column or row followed by the same superscript are not significantly different at P=0.05

Table 4.2. Sugar and starch levels in leaves and roots of 5 month old seedlings at various growth phases. Values are expressed as mg. Glucose equivalents per g. dry matter.

| Growth Phase | Plant Part | | | | | | |
|---|---|---|---|---|---|---|---|
| | Flush leaves | Top leaves | Bottom leaves | Roots | Mean | SED | L.S.D (5%) |
| (a) Reducing Sugars | | | | | | | |
| Dormant | | 37.84 | 35.59 | 25.54 | 32.99 | | |
| Bud Break | | 7.76 | 33.92 | 13.72 | 18.47 | | |
| Flushing | 48.38 | 22.44 | 15.16 | 15.1 | 25.27 | | |
| Mean | 48.38$^{c}$ | 22.68$^{ab}$ | 28.22$^{b}$ | 18.12$^{a}$ | | 8.13 | 9.96 |
| S.E.D | | | | | 8.06 | | |
| (b) Non-reducing Sugars | | | | | | | |
| Dormant | | 15.34 | 18.38 | 21.65 | 17.79$^{a}$ | | |
| Bud Break | | 20.74 | 25.03 | 26.69 | 24.15$^{b}$ | | |
| Flushing | 26.36 | 12.98 | 8.12 | 13.64 | 15.25$^{a}$ | | |
| Mean | 26.36 | 16.35 | 16.51 | 20.66 | | 4.75 | |
| S.E.D | | | | | 2.83 | | |
| L.S.D (5%) | | | | | 6.38 | | |
| (c) Starch | | | | | | | |
| Dormant | | 72.31 | 211.19 | 63.67 | 115.75 | | |
| Bud Break | | 79.63 | 131.83 | 74.09 | 95.18 | | |
| Flushing | 233.74 | 138.96 | 140.94 | 65.57 | 144.80 | | |
| Mean | 233.74$^{c}$ | 96.97$^{a}$ | 161.32$^{b}$ | 67.81$^{a}$ | | 23.36 | 49.53 |
| S.E.D | | | | | 32.09 | | |

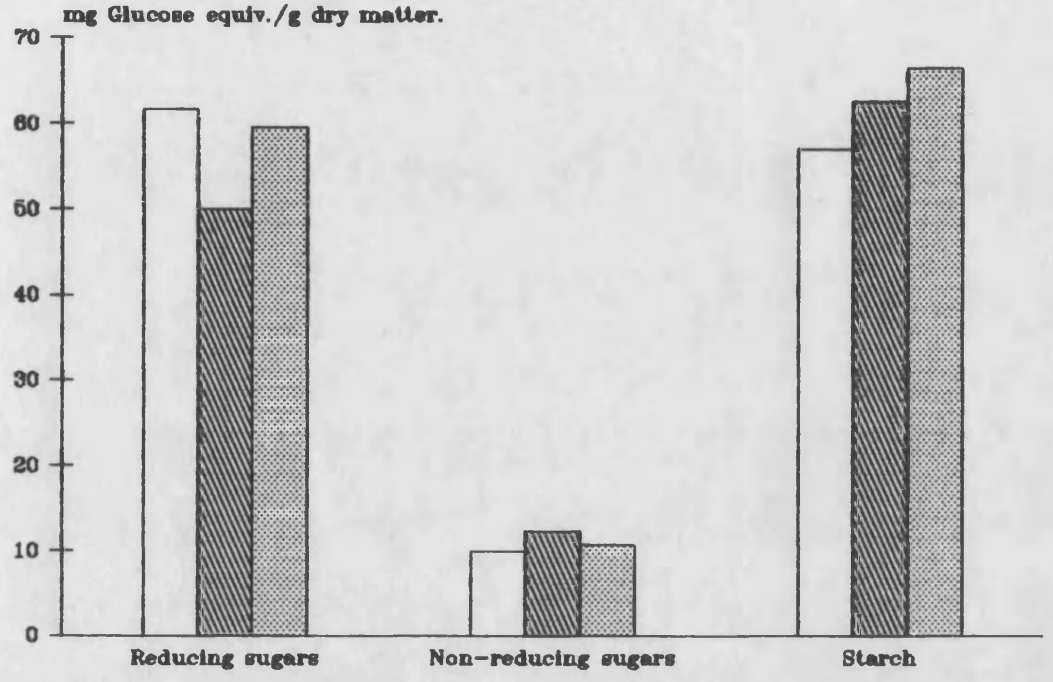
Means in the same column or row followed by the same superscript are not significantly different at P = 0.05.

**Fig.4.1 Levels of Total Non-structural carbohydrates (TNSC) at three growth phases of 3 and 5 month old seedlings.**

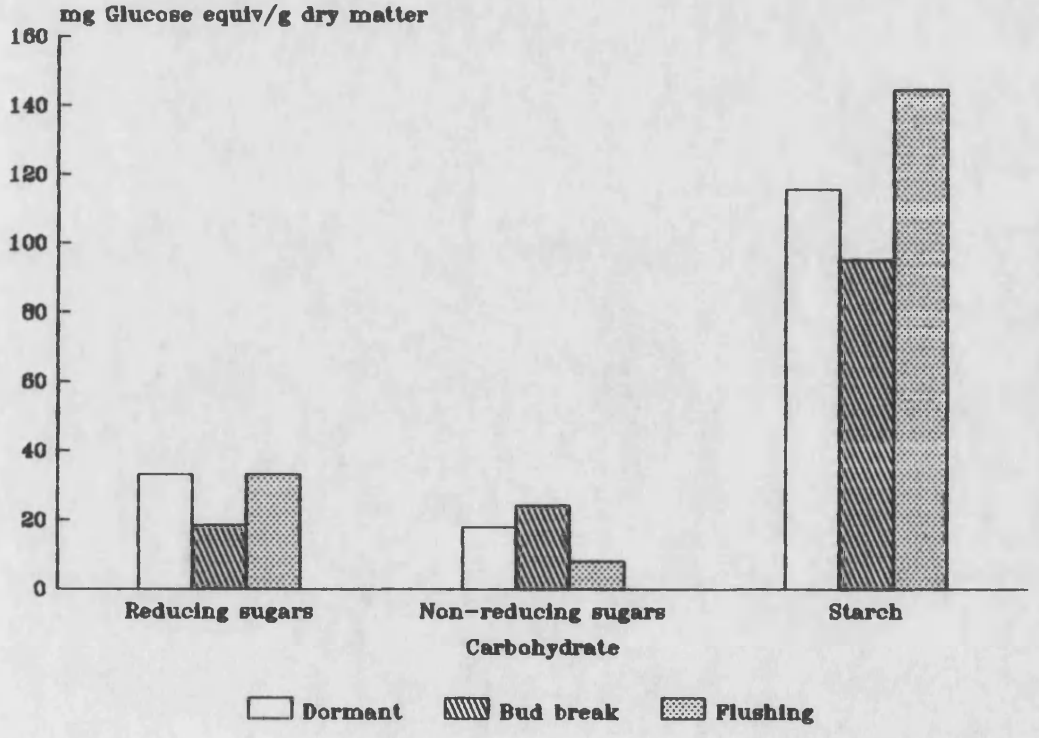



TNSC expressed as Glucose equivqlents per g. dry matter.

**Fig. 4.2 Mean plant carbohydrate levels in (a) three and (b) five month old seedlings at 3 growth phases**

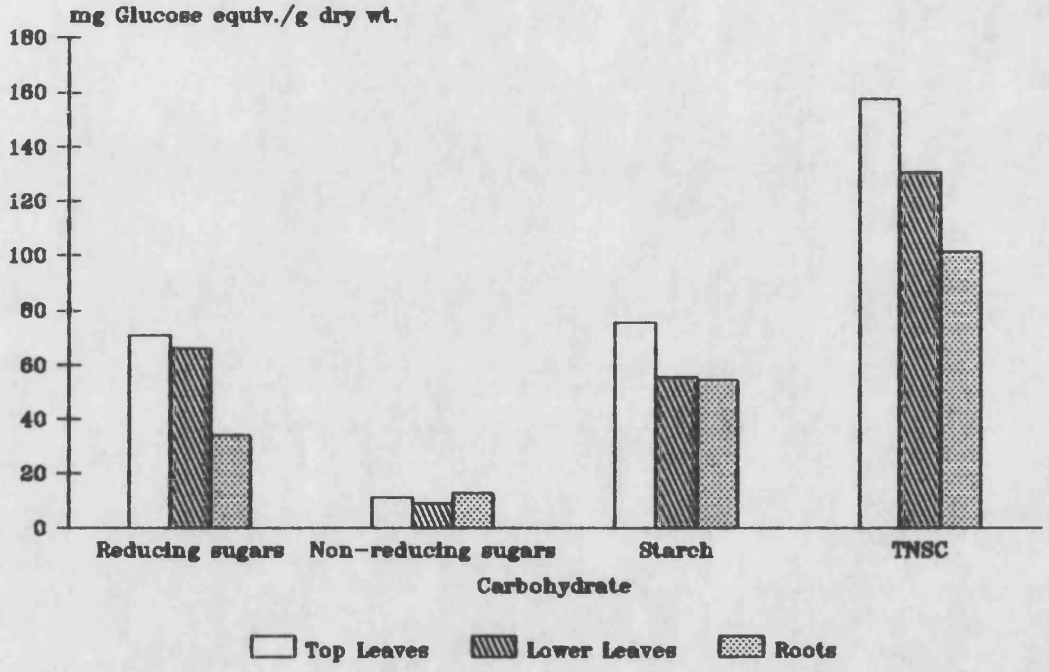



(a) Three months

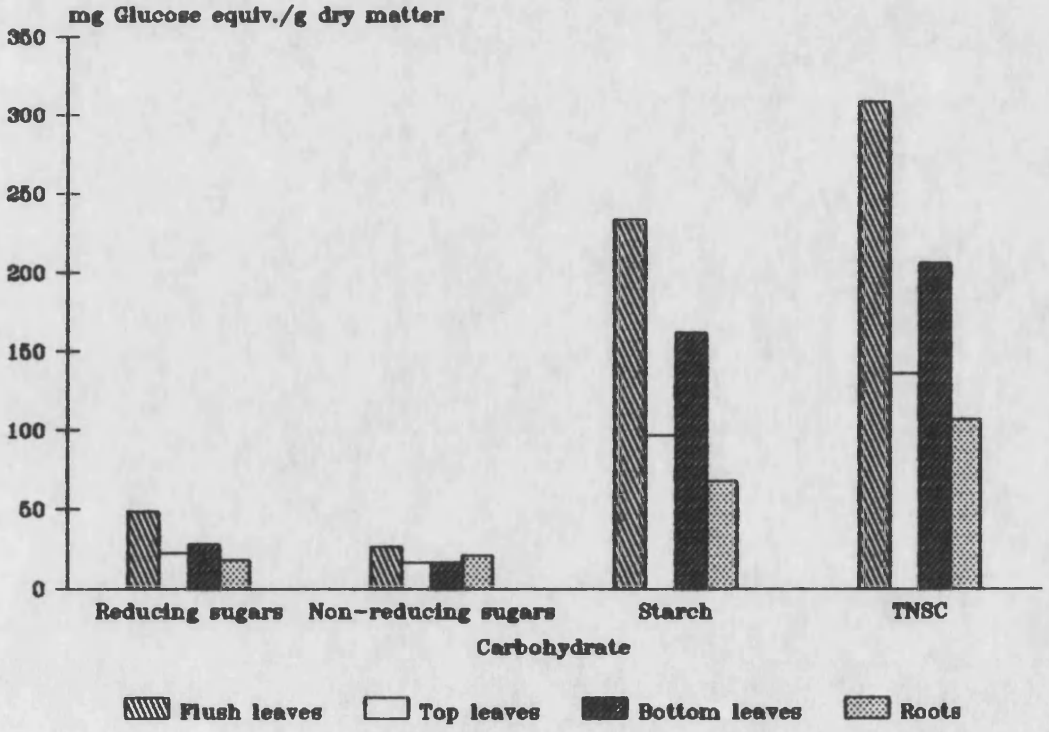



(b) Five months

**Fig. 4.3 Carbohydrate levels in leaves and roots of (a) three, and (b) five month old seedlings**




(a) Three months



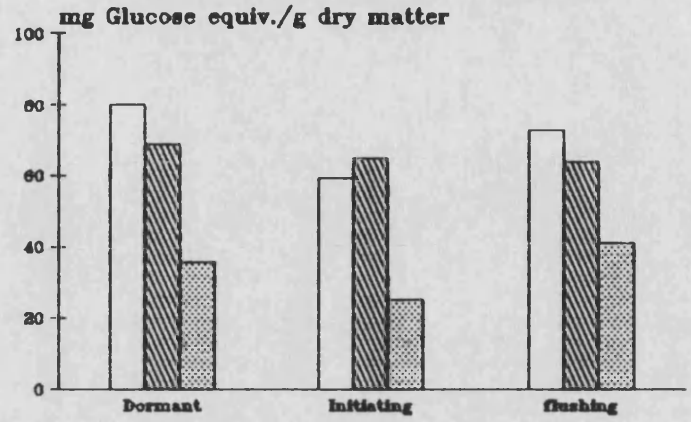

(b) Five months

leaves. In terms of total carbohydrates, flush leaves had one and half, half, and two and half times the concentration of top hardened leaves, bottom leaves and roots respectively.

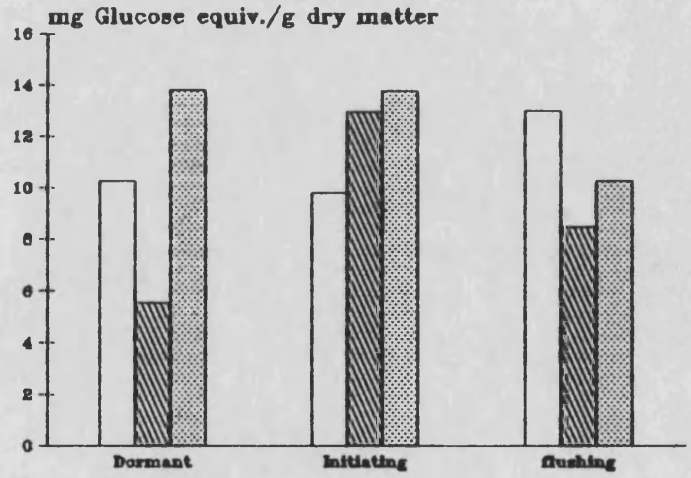
In dormant younger plants, top leaves (hardening) had the highest reducing sugar levels with lower levels in the hardened lower leaves and lowest in roots (Fig. 4.4a). In initiating plants, the top leaves (fully hardened) had lower reducing sugar levels than lower leaves. In flushing plants the actively expanding flush leaves had higher reducing sugars than the hard lower leaves and roots. At all phases of growth roots had the least reducing sugars. However, non-reducing sugar levels were highest in roots in both dormant and initiating plants (Fig. 4.4b). In flushing plants the flush leaves had higher levels. Top and flush leaves had the highest starch levels at all phases of growth (Fig. 4.4c).

In older seedlings, top and bottom leaves of dormant plants had similar reducing sugar levels. In initiating plants bottom leaves had higher reducing sugars than either roots or top leaves. In flushing plants the flush leaves had the highest levels (Figs. 4.5a). The trend is similar to that obtained with younger plants. Again roots had the highest non-reducing sugars in both dormant and initiating plants (Fig. 4.5b). In flushing seedlings, however, flush plants had the highest levels. In terms of starch, bottom leaves of both dormant and initiating plants had the highest starch levels, while roots and top leaves had similar levels (Fig. 4.5c). In flushing plants the flush leaves had the highest starch concentration with both top and bottom leaves having same levels and roots the lowest.

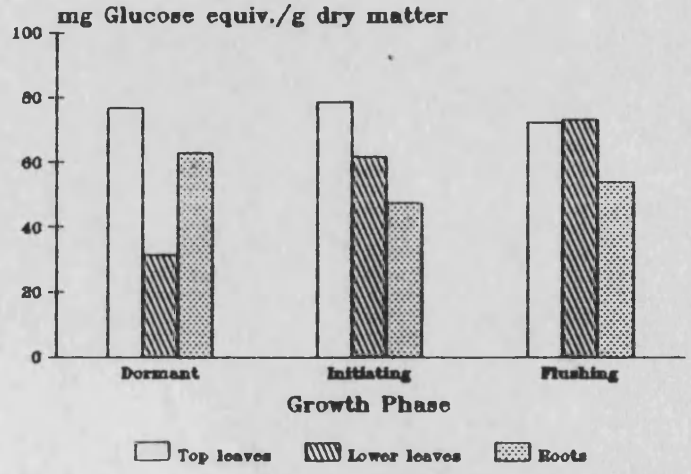
**Fig. 4.4 Levels of (a) Reducing, (b) Non reducing sugars and (c) Starch at three growth phases of 3 month old seedlings.**




(a) Reducing sugars

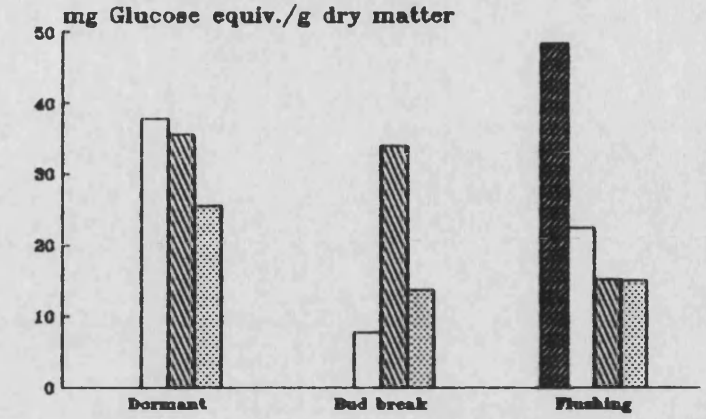



(B) Non-reducing sugars

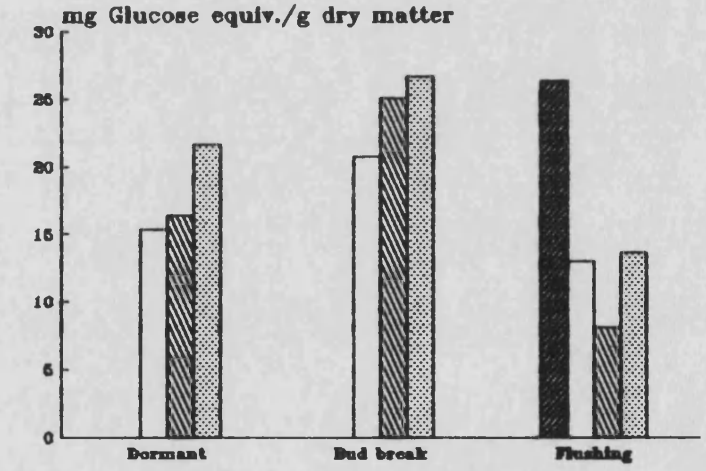



(C) Starch

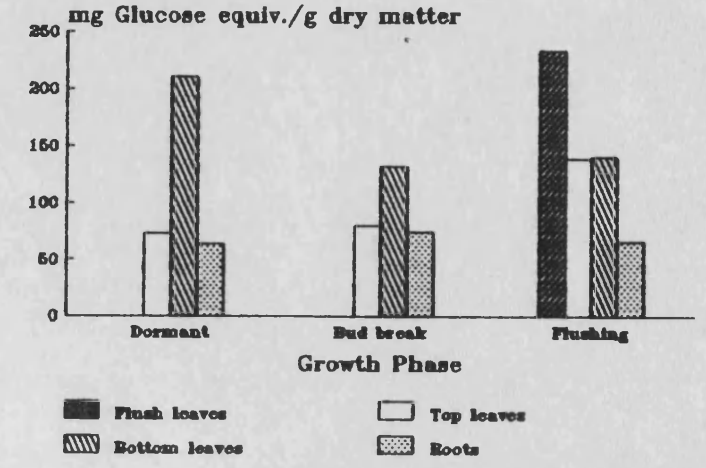
Fig. 4.5 Levels of (a) Reducing, (b) Non reducing sugars and (c) Starch at three growth phases of 5 month old seedlings




(a) Reducing Sugars




(b) Non-reducing sugars




(c) Starch

## 4.4. Discussion

The results show clear differences in concentrations of total non-structural carbohydrates between the various growth phases. The levels are lowest at bud break or in initiating plants and reach a peak in flushing seedlings. The presence of low levels at bud break is in agreement with work done by Abod, Webster and Quinlan (1991) who reported a decline in the total plant carbohydrate content of Tilia seedlings following bud burst. It is quite evident that the increase in carbohydrate concentration in flushing plants is largely due to high levels in flush leaves. There is also a clear pattern of decreasing concentrations down the plant in the younger plants.

In the 5 month old plants flush leaves had the highest TNSC levels while leaves immediately below (top mature) had TNSC levels lower than bottom leaves. This is apparently a result of carbohydrate mobilisation between various plant parts during growth. Sleigh, Collin and Hardwick (1984) have reported heavy mobilisation and translocation of carbohydrates from mature leaves, roots and stems to flush leaves during their period of expansion. This probably explains the low levels of carbohydrates in mature leaves nearest the flush here as they act as a source for the heavy sink of flush leaves. Baker and Hardwick (1975) and Owusu, Adamako and Hutcheon (1978) agree that expanding new flush leaves have very low photosynthetic capacity and are therefore not self sufficient in carbohydrates until they approach maximum size, an indication that for the major part of their development they are net importers for assimilates.

There are also some quite clear differences in the concentration of soluble carbohydrates and starch between the various growth phases and seedling ages. Reducing and non-reducing sugars represent the soluble carbohydrate pool which is immediately available for translocation and metabolic activities while starch represents the pool that can be mobilised for growth and maintenance processes. In general, macadamias have low non-reducing sugars relative to both reducing and

starch levels. In the younger plants the reducing sugar and starch concentrations were similar while in the older plants the starch concentration was 5 times higher than reducing sugars. This is perhaps an indication that the plant is building up considerable reserves for further growth and development. In the younger plants where growth flushes are more frequent a build up of reserves may not be possible. This is also perhaps reflected in that the older seedlings had higher non-reducing and twice the starch levels of younger plants.

Data on concentration of starch and soluble carbohydrates in the various plant parts during the three growth phases shows some rather interesting patterns. It should be pointed out that no fair comparison can be made between lower leaves of 3 month old seedlings and the bottom leaves of the older plants as the former were sampled from all leaves below the first pair while the latter comprise of the bottom pair on each plant. In the younger plants there is a reduction in reducing sugar concentration in top leaves and roots following bud break while levels in lower leaves do not show any changes. The pattern is more or less the same in older plants but there is a high level of reducing sugars in the flush leaves followed by a decline in bottom leaves. This perhaps suggests that the increase in reducing sugars in top leaves in the flushing phase is a result of mobilisation of the same from roots and bottom leaves.

Taylor (1988) reported very high reducing sugar concentration of individual flush leaves during early stages of the flush cycle in cocoa seedlings when leaves were 2 cm in length. As leaves began to expand the concentration decreased dramatically. In macadamia seedlings, there was also a decline in non-reducing sugars in other parts of the plant apart from flushing leaves. Starch seems to be stored mostly in mature bottom leaves and a high concentration is present in flushing leaves. Root starch levels remained the same at all growth phases. This perhaps indicates that the root reserves may not be necessary for flush growth in the young macadamia plants. This is contrary to findings by Abod et. al. (1991) who reported a decline in soluble sugars,

starch and total carbohydrates in roots of Betula and Tilia following bud break. They suggested that the decline was as a result of the translocation of the carbohydrates to the above ground organs to support growth. It should be noted that in these species root carbohydrate represented over 50% of total plant carbohydrate, hardly the sort of levels in macadamias.

The overall carbohydrate levels in the macadamia seedlings in the present study compare well with those reported elsewhere. The reducing and non-reducing sugar, and starch levels (expressed as mg glucose equivalent/g dry weight) are similar to those reported in seedlings of pecan (Carya illinoensis) by Wood (1984). The distribution of the sugars in the various plant parts, however, is not the same. The stem and tap root in the pecan were reported to have the highest starch and sucrose levels, while leaves had the highest reducing sugars. In the macadamia, leaves have had the highest of the three components and roots the lowest, except for sucrose. Of course the macadamia does not have as big a tap root as the pecan and no determinations were made on stem tissue. The starch levels reported in pecan tap root are extremely high, up to 4 times those of leaves. In the macadamia the roots do not seem to be able to store up as much starch, this is probably because macadamias have a weaker root system with shoot:root ratios of up to 3.7 compared to the pecan whose average ratios are less than 1. It is, therefore, suggested that the macadamia root may not play as significant a role as a source of carbohydrates during growth as reported for other crops such as pecans (Wood, 1984) and Tilia and Betula (Abod et. al., 1991).

The levels of starch in macadamia are similar to those reported in coffee (Patel, 1970). Coffee leaves at the height of their season had up to 9% starch which compares well with the levels in expanding leaves of macadamia. The total non-structural carbohydrate (TNSC) levels in this study are slightly higher than those reported by Cormack and Bate (1976) also working on macadamia. The maximum values here

have been 18% in flush leaves of 5 month old seedlings compared to 11.3% reported by Cormack and Bate (1976). This difference is due to several reasons including the methodology used and plant age. In this study TNSC's were derived from the component sugars of each sample from seedlings, while Cormack and Bate (1976) conducted direct determinations of TNSC on samples from field trees. They reported high levels of TNSC's in fully matured non-flushing macadamia shoots followed by a progressive decrease as flush is initiated up to hardening. In the case of seedlings here, flushing shoots had the highest total carbohydrates. The discrepancy can be explained by taking into account reproductive growth in the field trees. Flushing occurs while nuts are in production and the combined demand of flush and nut development may lead to the low levels while in seedlings there is no such competition. Indeed Stephenson et. al. (1989) have advocated that the macadamia crop is a stronger sink than the shoots.

The reducing sugar and starch levels are comparable to those reported in cocoa by Taylor (1988), while those of sucrose are much lower in the macadamia. In the macadamia it is evident that the topmost leaves, which were expanding but longer than 20cm, also had higher reducing sugar and starch levels than lower ones. The high sugar levels in younger leaves could be due to several reasons. Our previous research has shown that macadamia leaves develop very rapidly which might mean that they quickly turn from a heavy sink to a source. Quite simply the young leaves could be more efficient photosynthetically, or they are such strong sinks that there is heavy mobilisation of reserves from the older portion. The latter seems more plausible. Also it is possible that shading of older portions by the new flush could reduce their photosynthetic potential. The feature of more carbohydrates in top leaves and presumably the young wood upon which they grow may partly explain the growth flush pattern exhibited by macadamia seedlings with numerous flushes in some instances with hardly a pause between them. The terminal parts of seedlings are

invariably in an immature phase most of the time and could be presumed to have fairly high carbohydrate levels.

CHAPTER 5.

# EFFECTS OF PACLOBUTRAZOL ON THE GROWTH AND CARBOHYDRATE DISTRIBUTION OF MACADAMIA SEEDLINGS.

## 5.1. INTRODUCTION

### 5.1.1. Paclobutrazol

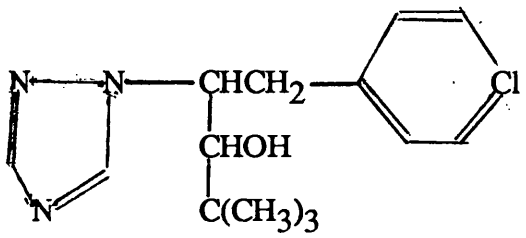
The appreciation of the role of phytohormones as endogenous substances that regulate plant growth and development has led plant scientists to apply chemicals exogenously to crops to direct their productivity by other than nutritive means. Several chemicals are now widely used to promote or delay ripening and senescence, induce flowering, control abscission, and promote or retard growth (Tukey, 1981; Quinlan and Richardson, 1984; Monselise, 1986; Shearing and Jones, 1986)). These chemicals are collectively referred to as plant growth regulators, one of which, paclobutrazol, has been used in the present research on macadamia. Paclobutrazol (PBZ) was first synthesized by the Imperial Chemical Industry (I.C.I) in 1976 (Froggart, Thomas and Batch, 1981). It is a growth retardant and inhibits plant vegetative growth and reduces plant vigour.

### 5.1.2. Structure and Mode of Action

Paclobutrazol: (2RS, 3RS)-1 -(-4-chlorophenyl)-4,4-dimethyl -2-(1H-1,2,4 triazol-1-yl) pentan -3-ol, also called PP333 or CULTAR.

empirical formula; $C_{15}\ H_20\ Cl\ N_30$

structural formula;




Structurally it is a substituted triazole with two asymmetric carbon atoms and produced as a mixture of the 2R, 3R and 2S, 3S enantiomer. Functionally

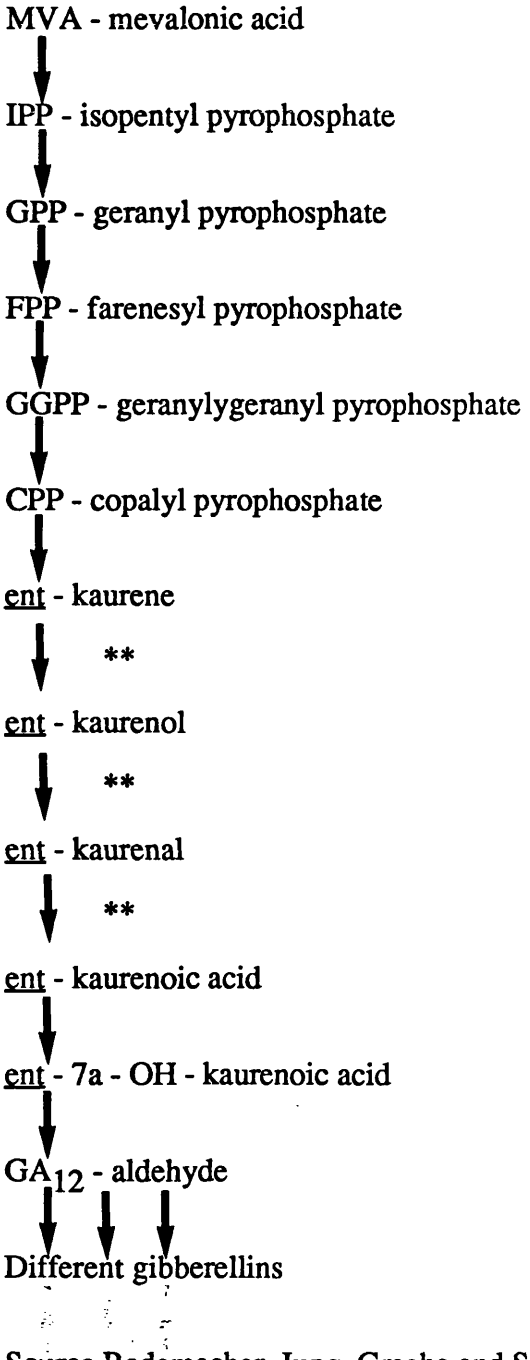
paclobutrazol is a broad spectrum growth retardant formulated as a suspension concentrate, wettable powder, or granules and is water soluble (35 mg/l) with low mammalian toxicity. The acute and dermal LD50 values to the rat are approximately 1500 and 1000 mg/kg respectively. It produces dose related reductions in vegetative growth without scorch or phytotoxicity (Lever, Shearing and Batch, 1982).

The major biochemical effect of paclobutrazol is the inhibition of gibberellin (GA) production by blocking the oxidation of kaurene to kaurenoic acid in the GA biosynthesis pathway (Fig. 5.1). This has been demonstrated in cultures of Gibberella fujikuroi (Goldsmith, Hood and MacMillan, 1983), cell free systems from pea apices (Dalziel and Lawrence 1984), and in Cucurbita maxima endosperm (Hedden and Graebe, 1985). This mode of action is similar to that of other nitrogen-containing heterocyclic growth retardants such as ancymidol and tetcyclasis which have been shown to inhibit gibberellin biosynthesis by blocking specifically the three steps in the oxidation of the gibberellin precursor ent-kaurene to ent-kaurenoic acid (Coolbaugh, Hirano and West, 1978; Rademacher, Jung, Graebe and Shwenen, 1984). Apparently paclobutrazol does not inhibit the activity of existing endogenous GA, nor of exogenously applied GA. The finding that paclobutrazol inhibits $GA_3$ biosynthesis is further supported by those reports that its effects, and that of other triazoles, can be alleviated almost completely by a simultaneous application of $GA_3$ (Rademacher et. al., 1984; Casper and Taylor, 1989).

### 5.1.3. **Uptake and Translocation**

Paclobutrazol may be taken up passively through roots, stem tissue and foliage. Within the plant, movement is acropetal, moving exclusively in the xylem (Lever, 1986). It is believed to move slowly to active sites in sub-apical meristems from reservoirs in soil or stem tissue, and can also be taken up through young sub-apical shoot tissues following foliage sprays (Quinlan and Richardson, 1984). There is no evidence that paclobutrazol can move in the phloem. Studies with $C^{14}$-labelled

Fig. 5.1 Pathway of gibberellin biosynthesis showing the steps blocked by the tetcyclasis and triazole type retardants (**)




Source Rademacher, Jung, Graebe and Schwenen (1984)

paclobutrazol (Richardson and Quinlan, 1986) showed no phloem mobility and chemical absorbed by leaves was not exported to adjacent shoots or leaves, even before leaf fall when some leaf contents can be remobilised.

Since the retardant is almost exclusively transported through the xylem, soil applications may be more effective than foliar sprays in retarding growth (Williams and Edgerton, 1983; Erez, 1984; 1986). However, several studies have indicated that repeated foliar applications may be as effective as single soil applications (Tukey, 1983; Webster and Quinlan, 1984; Shearing and Jones, 1986). Differences in the effectiveness of PBZ due to site of application are probably due to the differences in the ability of the compound to be translocated in the xylem and phloem. Movement of paclobutrazol, applied through the soil, to the shoot apex from the roots and sites along the stem would be via the transpiration stream in the xylem. Chemical taken up by the leaves, however, would have to move through the phloem at least to the stem where it might be transferred to the xylem.

Thus the reduced effectiveness of PBZ applied to mature leaves suggests poor translocation through the phloem compared to the xylem, requiring metabolic energy for the transfer from the free spaces into the living parts of the cell and to move material along the symplastic pathway (Barret and Bartuska, 1982). Paclobutrazol applied as a foliar spray may be absorbed by both the leaves and stems but only in the latter is it effectively translocated to its site of action in the shoot apex, probably via the xylem.

The efficiency of utilisation of soil applied material is determined by factors which influence passive movement in the soil and tree. The extent of the movement of paclobutrazol in the soil depends upon soil water movement and the absorption coefficient in the particular soil type. Since the retardant is relatively immobile, its uptake through roots is critically dependent upon the juxtaposition of chemical and

roots. The most efficient uptake will occur when chemical and root are concentrated in the same small area. Foliage cover and an active transpiration stream are then required to pull the chemical up to the growing points.

### 5.1.4. The Use of PBZ

Paclobutrazol is active on a wide range of mono and dicotyledonous plants and its effects have been reported on a wide range of species ranging from graminaceous crops to fruit trees (Dalziel and Lawrence, 1984). The potential benefits include: control of tree vigour and enhancement of fruit bud development; reduction in pruning requirements; better light penetration resulting in improved fruit quality and colour; and increased frost tolerance.

Early reports associating paclobutrazol with inhibition of extension growth in trees were published by Quinlan (1980) following work on Bramley seedling apples. Later, Stinchcombe, Copas, Williams and Arnold (1984) reported significant reductions in shoot growth as a result of soil applications of the retardant to cider apple trees. Growth was markedly reduced, leaf area was also reduced and laminae became darker in colour. High rates of PBZ resulted in more secondary compensatory shoot growth during the latter part of the season. More research by Quinlan and Richardson (1984) showed that PBZ applied to apple shoot stems and tips was more effective in controlling shoot growth. Soil applications were found to be rather ineffective, while foliar applications were very effective.

Quinlan and Richardson (1984) also found that effects of paclobutrazol could be completely overcome by the application of gibberellins, specifically $GA_3$. This implies that some control on tree growth could be achieved through use of the two chemicals. This contention was strengthened by Steffens and Wang (1984) who reported 91% reduction in shoot length, 66% reduction in leaf area, and 17%

reduction in leaf number following continual applications of the retardant to apple seedlings. Foliar applications of $GA_3$ restored leaf area more effectively than it did longitudinal shoot growth. Marquard (1985), working on pecan seedlings, also reported reductions in tree height, stem weight, and increased root:shoot ratios following paclobutrazol applications. It was found to be more effective than fluriprimidol, another growth retardant. However, use of promalin (which contains both $GA_3$ and BA) induced shoot growth of some terminal and lateral buds and promoted shoot elongation. On peach seedlings, paclobutrazol reduced current season's extension shoots and lateral shoots by 80% compared to control over a 15 week period (Liyembani and Taylor, 1989) and Steffens and Wang (1984) reported decreases in leaf area by 50% and stem length by 85% after 21 days following application in apple seedlings.

The timing and method of application have been reported to influence effectiveness of paclobutrazol. The most effective application methods are dependent on crop species and growth patterns. Generally, foliage sprays are more effective than soil applications on pome fruits (Quinlan, 1981), while both soil and foliar applications are effective on stone fruits. In apples, sequential sprays at a reduced rate have proven more effective than a single higher dose spray (Lever, 1986). This is apparently because the paclobutrazol reservoir can be more effectively replenished from exogenous application than internal translocation.

In pecans, trunk injection and foliar sprays of paclobutrazol to 75 year old trees resulted in reductions in terminal shoot growth and leaf area during 4 years following treatment (Wood, 1988). However, trunk injection resulted in loss of nut production in the third and fourth years, which was probably due to reduced tree assimilate levels as a result of a huge decline in individual leaf area and leaves per shoot with increasing retardant levels. Blanco (1988) reported that soil applications of paclobutrazol in early spring reduced shoot extension in 6 year old nectarine and

peach trees, with the effects increasing linearly with amount applied. Autumn soil applications were less effective and single foliar applications were not effective at all. There was also a difference in response by various cultivars indicating differential cultivar sensitivity to the chemical.

The use of growth retardants in intensively managed orchard systems offers several advantages. Synergistic interactions between tree growth at different spacings and paclobutrazol have been reported by Quinlan (1988). At the closest spacing, apple tree growth was reduced three times more severely than at the widest spacing. Although all trees received sprays of the same concentration, it is likely that the densely planted parts of the trial received a higher amount of chemical per unit area of orchard resulting, eventually in a greater uptake of the growth regulator.

Paclobutrazol has also found use on apple trees grafted on to dwarfing rootstocks. Usually such trees tend to be poorly anchored and each tree has to be supported by a tall stake, the cost of which usually exceeds 20% of total establishment (Webster and Quinlan, 1986). Application of PBZ to closely planted trees (750 trees/ha) on MM106 rootstock has enabled free-standing trees of this combination to be grown at a spacing conventionally used for Bramley apple on M9 rootstock which has to be grown with a heavy stake.

Regulation of tree growth is an acute problem with crops such as sweet cherry for which no dwarfing rootstock is yet available. The problem of large tree size has resulted in the decline in cherry growing in several European countries despite unsatisfied consumer demand for the fruit. Webster and Quinlan (1986) have reported marked reductions in canopy volume of sweet cherry following applications of paclobutrazol for four years. Yield per tree was reduced but cropping efficiency in terms of yield per unit canopy volume was increasing, suggesting that this treatment

may be used to increase sweet cherry productivity by growing trees at close spacings while relying on chemical growth regulation to contain tree growth.

### 5.1.5. Effects of paclobutrazol

The main biological effect of paclobutrazol is to inhibit vegetative growth of the plant. Associated with this is the alteration in sink strength within the plant, allowing a greater proportion of assimilates to contribute to reproductive growth including flower bud formation and fruit growth. The most marked morphological effect of paclobutrazol is a dose-related reduction in internode length on terminal and lateral shoots. Treated trees also tend to produce more flower buds, flowers and fruit numbers as a consequence of the previous season's treatments.

Paclobutrazol has been associated with distribution of dry matter and partitioning of carbohydrates within the plant. Steffens and Wang (1984) and Steffens, Byun and Wang (1986) reported on dry matter distribution in apple seedlings following treatment with paclobutrazol. Compared to control plants, the retardant reduced the weight of new leaves by 38%, new stems by 66% but increased the weight of fibrous root by 61%. Fibrous root length was increased by 152%. Relative chlorophyll content on a leaf area basis increased in treated plants.

Further research (Wang, Byun and Steffens, 1985) has shown effects of paclobutrazol on carbohydrate partitioning in apple seedlings. The chemical increased the carbohydrate concentrations in all parts of the seedlings. Treatments resulted in increases of sorbitol by 25% in original stems, 36% in new leaves, 40% in tap roots and 70% in new stems. Starch accumulation in old stems and leaves was enhanced even more. The partitioning of assimilates was altered with carbohydrates being increased in the roots, and reduced in leaves, whilst soluble protein content was increased in leaves. In mature trees (Steffens, Byun and Wang, 1986) sorbitol was the major carbohydrate in the shoot and fruiting spur leaves following application of

paclobutrazol. Sucrose was not affected by treatment and remained relatively low in both leaf types, but starch was significantly increased.

The primary biochemical effect of inhibition of gibberellin biosynthesis is the reduction in GA levels which results in reduced rates of cell division. Bayliss, (1984) reported low cell division rates following PBZ application to single cell cultures of sugar beet. There were indications of rapid reduction in numbers of mitotic and S-phase nuclei in treated cultures. However, cell volume was not affected and inhibited cultures had greatly enlarged cells.

Paclobutrazol has also been reported to affect photosynthesis. Lawrence (1984) reported higher rates of photosynthesis on treated apple leaves, per unit leaf area, at all light intensities. Treated leaves reached carbon dioxide saturation at lower concentrations than controls. This was attributed to higher internal surface area and higher chlorophyll levels in leaves treated with paclobutrazol. While the retardant has been reported to reduce leaf area, leaf thickness and chlorophyll content are increased (Jaggard, Biscoe and Lawrence, 1982). Freeze electron microscopy has shown that elongation of mesophyll cells, especially those of the palisade mesophyll, is responsible for increased thickness. This elongation contributes to the increase in the internal surface area of the leaf and increased photosynthesis. Plants treated with paclobutrazol have also been reported to exhibit reduced water use levels (Wample and Culver, 1983), but this is primarily due to the reduced leaf area rather than to any physiological changes.

### 5.1.6. Aim of study

The problem of excessive vegetative growth and its effects on the efficiency of crop production in macadamia has already been discussed (Chapter 1.2). It was, therefore, considered that paclobutrazol (PBZ) could be used, not only in controlling vegetative growth but also in the partitioning of assimilates between vegetative and reproductive

growth. The series of experiments in this section were aimed at investigating the effects of PBZ on the growth and development of macadamia seedlings and its effects on carbohydrate distribution within the seedlings.

## 5.2. Materials and Methods

Experiment 1:. Effects of paclobutrazol on the growth and carbohydrate distribution of six month old seedlings.

Three month old seedlings growing in 2 litre plastic pots were treated with 0, 5, 10, 20, 40, and 80 mg active ingredient paclobutrazol per plant on 15 March 1990. Cultar (23% W/W paclobutrazol, ICI Agrochemicals, U.K) was used as the source of the retardant which was applied in 250ml solution onto the soil around each plant. 250ml of water were applied to controls. The experiment was laid out in a randomised complete block design with 6 blocks. Plants were grown on a middle bench in the glasshouse under conditions already described in Chapter 2.1.

Data on extension growth and leaf area were taken at fortnightly intervals for three months. At the end of the experiment, by which time seedlings were six months old, they were separated into roots, stems and leaves. The components were dried in a forced draught oven for 1 hour at $95^0$C followed by 4 hours at $70^0$C. The dried material was used for dry matter assessments and samples taken for carbohydrate determinations as already described in Chapter 2.3.

Experiment 2: Effects of paclobutrazol on the growth of seven month old seedlings.

This experiment was set up to determine effects of paclobutrazol on the growth of seedlings at graftable age. 4 month old seedlings growing in 2 litre pots were treated with the retardant as in Experiment 1 using 0, 5, 10, 20, 40, and 80 mg a.i per plant applied on 3 October 1990. This experiment was conducted as a double latin square with 6 blocks in each square. Data on growth were collected as in Experiment 1 for 3 months.

Experiment 3: Effects of low doses of paclobutrazol on the growth of eight month old seedlings.

Based on growth patterns observed in Experiment 1 this was set up to assess the effects of low doses of paclobutrazol on growth. Seedlings, at five months of age, were treated with the retardant at concentrations of 0, 0.5, 1, 2, 4, 8, 16, 32, and 64 mg a.i per plant on 8 October 1990 in an experiment laid out as a single latin square with 6 replicates. Data on extension growth and leaf growth were collected monthly for 3 months following application of the retardant.

## 5.3. RESULTS.

### 5.3.1. Effects of PBZ on extension and leaf growth.

The effects of PBZ on seedling growth were noticeable six weeks after application and persisted up to twelve weeks later (Appendix 2C(a)). Over this period, seedlings treated with high levels of PBZ remained stunted (Plate 5.1), their shoots had thickened stems and their foliage appeared rosetted. At the end of 9 weeks there was evidence of release from initial inhibition with treated plants producing growth flushes. Application of PBZ had profound effects on extension growth of seedlings at all ages used. Growth data over a three month growing period following PBZ application to three month old plants shows significant reductions ($P = 0.001$) in stem extension growth in plants treated with high levels of PBZ compared to untreated plants (Fig. 5.2 and Appendix 2C(b)).

Application of the highest PBZ levels resulted in the least extension growth with 20%, 55% and 67% shorter stems for plants receiving 20, 40, and 80 mg PBZ respectively compared to untreated plants. However, there were also significant increases in extension growth in plants receiving the lowest PBZ rate (5 mg). This was confirmed by results from Experiment 3 which evaluated the effects of low doses of PBZ on 8 month old seedlings (Fig. 5.3 and Appendix 2E). Growth over a period of three months following PBZ application at five months showed that although no significant differences in stem extension growth were obtained (largely because a narrow band of PBZ doses was used), trends in growth showed growth stimulation in plants receiving doses of between 0.5 and 4 mg PBZ. Compared to untreated plants, extension growth increases of 15%, 28% and 5% were obtained for plants receiving 0.5, 1, and 2 mg PBZ respectively. Higher PBZ levels resulted in reductions in extension growth.


Plate 5.1 6 month old seedlings treated with PBZ at 3 months of age. Note the stunted growth and rossetted appearance of the plants treated with higher PBZ levels. PBZ levels are **(a)** 0, **(b)** 5, **(c)** 10, **(d)** 20, **(e)** 40, and **(f)** 80 mg PBZ per plant.

**Fig. 5.2 Stem extension growth (mm) on 6 and 7 month old seedlings following PBZ application at 3 and 4 months of age.**

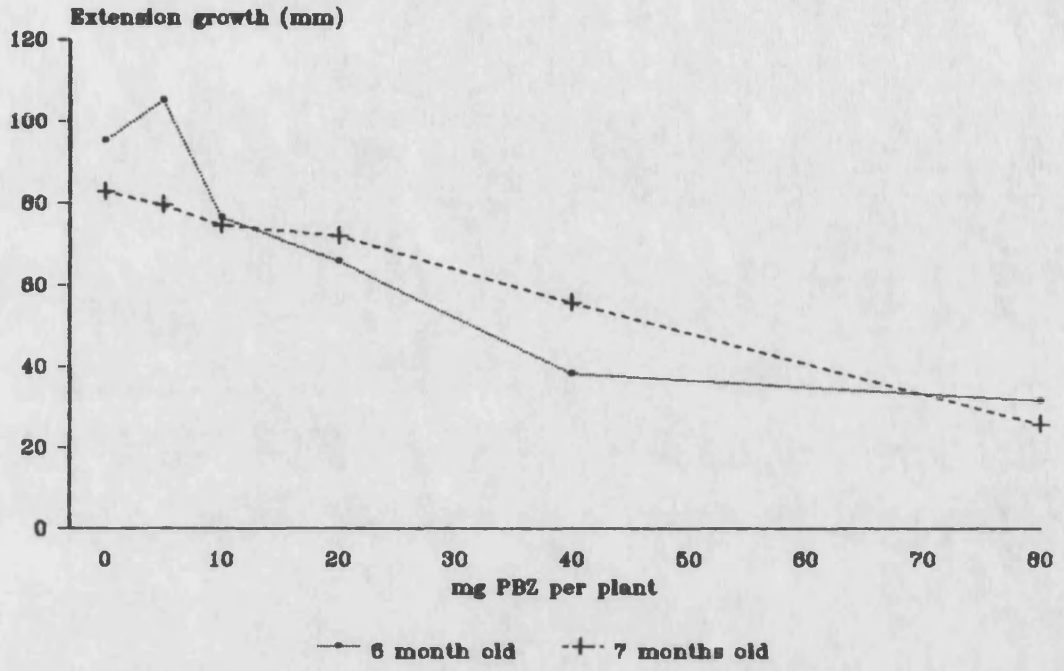
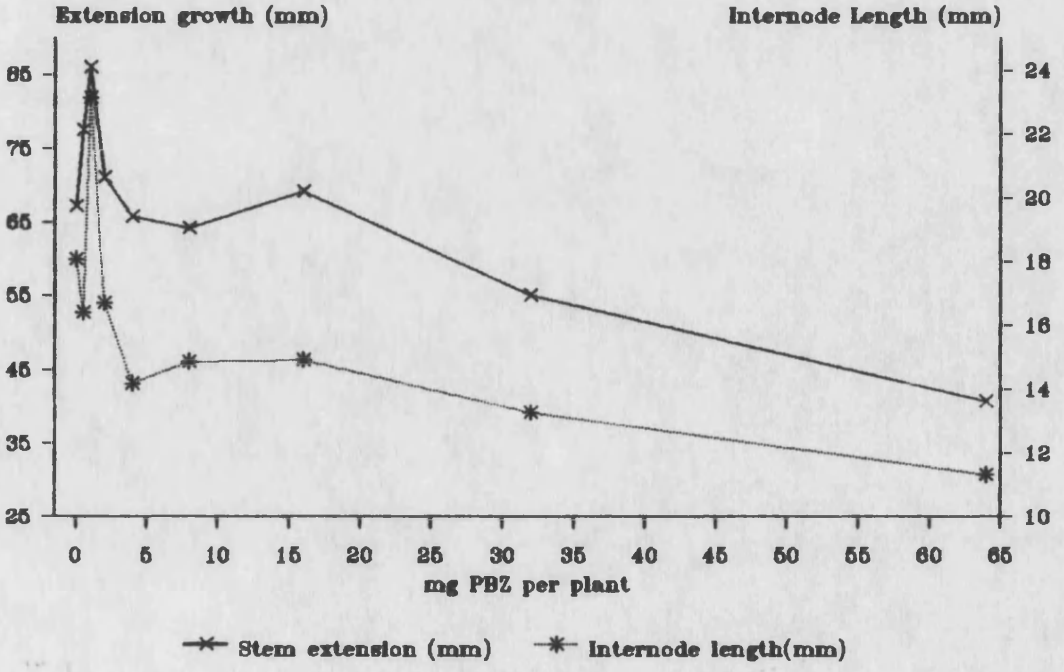



**Fig. 5.3 Stem and internode growth (mm) on 8 mth. old plants after application of low doses of PBZ at 5 months of age**



In Experiment 2, where PBZ was applied to four month old seedlings, growth over a period of three months showed significant (P = 0.05) dose-related reductions in extension growth (Appendix 2D). Plants treated with 80 mg PBZ had 68% less extension growth than untreated plants (Fig. 5.2), which is comparable to reductions obtained in Experiment 1 at the same rate of PBZ. In this case, however, there was no growth stimulation in plants receiving lower rates of PBZ.

There is evidence to indicate that stimulation or retardation of stem extension growth was as a result of effects on internode growth. Data on six month old plants (Table 5.1) and eight month old plants with low doses of PBZ (Fig. 5.3) show patterns of increases and reductions in internode length similar to those obtained in extension growth.

PBZ application had no significant effects on leaf area (Fig. 5.4 and Appendix 2E(b and c)). Although there were increases in leaf area in plants receiving low rates of PBZ, the increases were as a result of increased leaf production (Table 5.1) rather than due to increases in leaf sizes.

### 5.3.2. Effects of PBZ on dry matter distribution

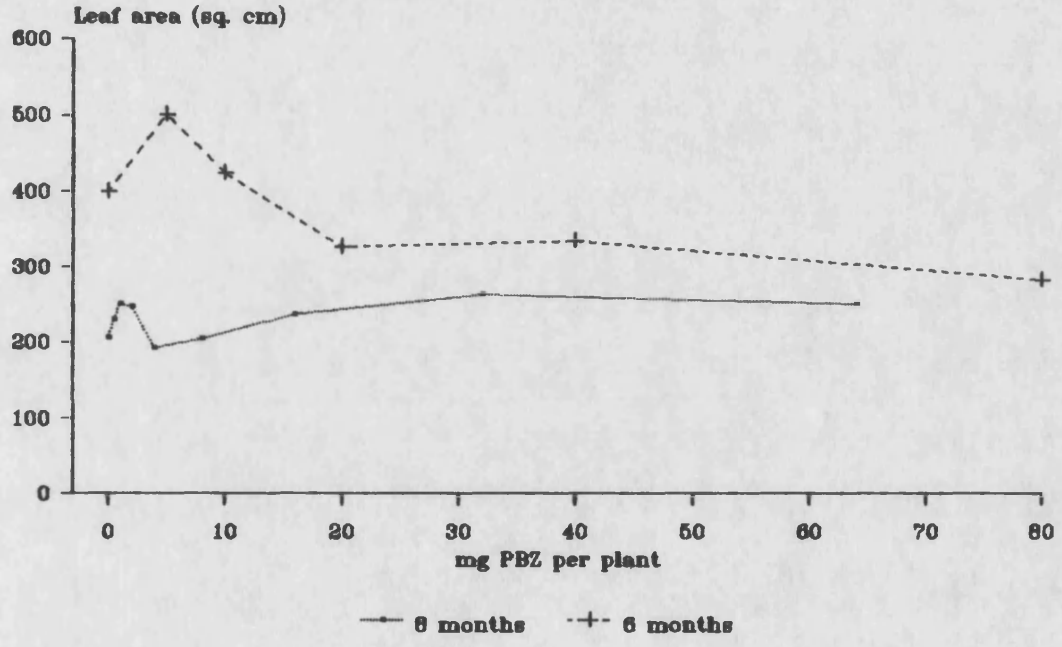
There were statistically significant differences (P = 0.05) in the distribution of shoot and root dry matter following treatment of three month old seedlings with increasing levels of paclobutrazol (Table 5.1). Measurements taken three months later show general reductions in total shoot dry weight with increased levels of PBZ. Plants receiving the lowest PBZ and those with no PBZ had the highest total shoot and root dry matter. There were also increases in leaf dry matter at the lowest PBZ rate, largely as result of the increase in number of leaves rather than increases in leaf size. The shoot:root ratio was maintained between 2.3 and 3.5. Although plants with the lowest PBZ had the lowest ratio, no general trend could be discerned from the data in this parameter.

Table 5.1 Growth and dry matter accumulation in 6 month old seedlings following treatment with various rates of paclobutrazol at 3 months of age.

| | mg. Paclobutrazol per plant | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Parameter | 0 | 5 | 10 | 20 | 40 | 80 | S.E.D(±) | 5% L.S.D |
| Total leaf wt.(g)/plant | $8.8^{cd}$ | $9.1^{d}$ | $8.2^{bc}$ | $7.7^{ab}$ | $7.9^{a}$ | $7.3^{a}$ | 0.28 | 0.68 |
| Total root wt.(g)/plant | $5.8^{c}$ | $5.8^{c}$ | $4.1^{b}$ | $2.9^{a}$ | $3.7^{ab}$ | $4.2^{b}$ | 0.48 | 1.17 |
| Number of leaves per plant. | $10.8^{bc}$ | $12.1^{d}$ | $11.0^{cd}$ | $11.3^{cd}$ | $8.6^{a}$ | $9.6^{ab}$ | 0.51 | 1.25 |
| Mean internode length (mm) | $22.2^{a}$ | $26.1^{c}$ | $21.7^{b}$ | $21.9^{b}$ | $21.8^{b}$ | $18.3^{a}$ | 1.01 | 2.47 |
| Total shoot wt (g) | $13.8^{e}$ | $13.6^{de}$ | $12.1^{cd}$ | $10.2^{ab}$ | $11.9^{bc}$ | $9.7^{a}$ | 0.21 | 0.52 |
| Shoot:Root ratio | 2.48 | 2.30 | 2.90 | 3.50 | 3.10 | 2.20 | | |

Means in the same row followed by the same superscript are not significantly different at P=0.05.

**Fig. 5.4. Increases in total leaf area (sq. cm) in 6 and 8 month old plant after PBZ application at 3 and 5 months**




8 month old seedlings treated at low doses of PBZ

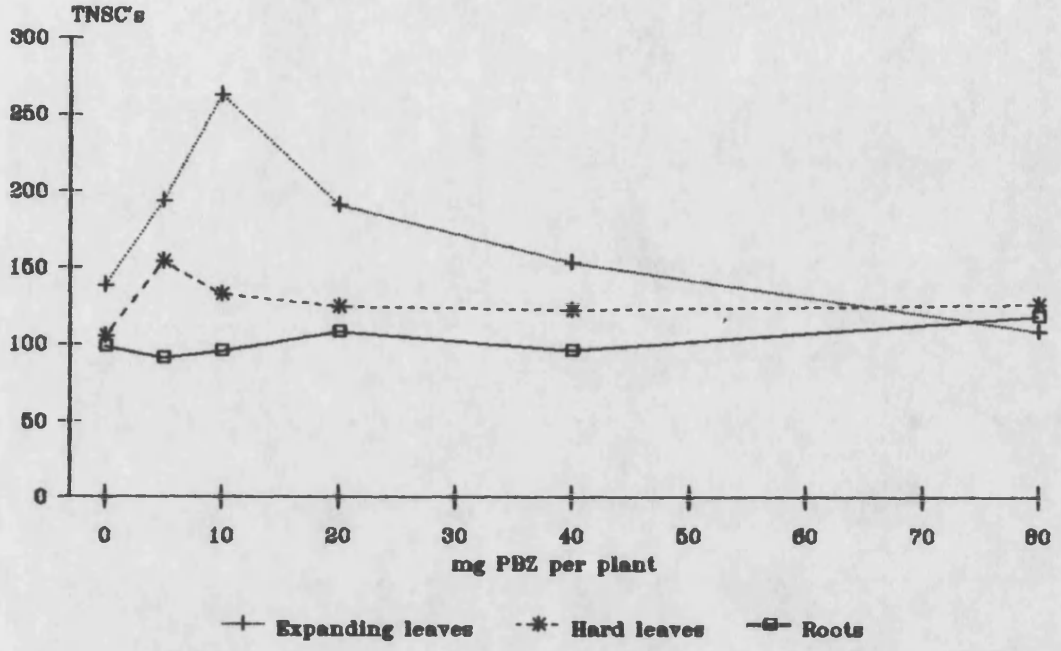
### 5.3.3. Effects of PBZ on carbohydrate distribution.

Carbohydrate determinations were conducted only on six month old plants following PBZ application at three months of age. The total non-structural carbohydrates (TNSC) were determined from totals of reducing and non-reducing sugars and starch components expressed as mg. glucose equivalents per g. dry weight. Both the overall levels and the distribution of reducing, non-reducing sugars and starch were affected by PBZ treatments. PBZ had no significant effects on the total carbohydrate distribution in the whole plant although the distribution of reducing sugars and starch in the various plant parts was significantly affected (Appendix 2C(ii)). TNSC levels in leaves were increased in plants which had received the two lowest PBZ rates (Fig. 5.5). Plants treated with 5 and 10mg PBZ had 30% and 43% higher TNSC levels than untreated plants respectively. Higher PBZ rates resulted in lower TNSC levels although the values were all higher than those of untreated plants. Plants receiving the 80 mg PBZ had the lowest TNSC levels of all the treated plants but still 8% higher than untreated plants.

In terms of distribution within the plant, TNSC levels were highest in expanding leaves and lowest in roots (Fig. 5.5). Plants treated with low PBZ levels had higher leaf TNSC levels than those treated with higher levels of PBZ. This was more pronounced in expanding leaves rather than mature hard leaves. There was not much variation in root TNSC levels at all rates of PBZ, although levels were lower at higher PBZ rates coinciding with the high leaf TNSC levels.

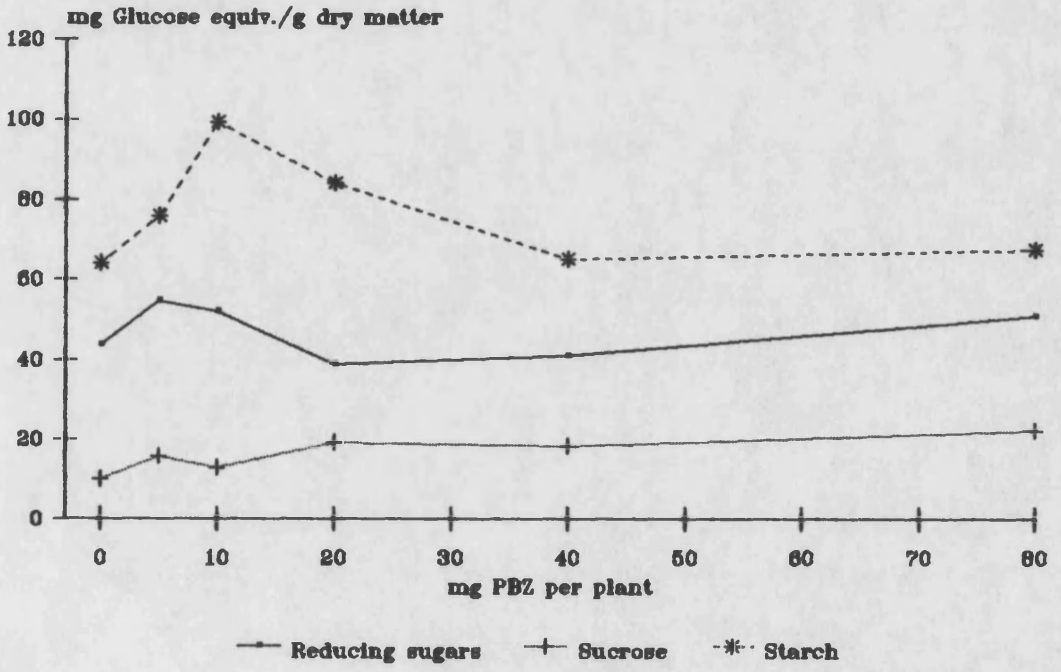
Starch formed the largest component of total plant carbohydrate (Fig. 5.6), while non-reducing sugar levels were the lowest component. Both starch and reducing sugar levels were higher in plants receiving the lowest PBZ levels (5 and 10mg a.i) compared to untreated plants and those receiving higher PBZ rates. Starch and sugar levels were not at all influenced with higher rates of PBZ.

**Fig. 5.5 Levels of Total Non-structural Carbohydrates (TNSC) in leaves and roots of 6 mth old plants treated with PBZ.**




TNSC's are expressed as mg Glucose equivalents per g dry matter.

**Fig. 5.6 Carbohydrate composition of 6 month old plants following treatment with PBZ at 3 months of age.**

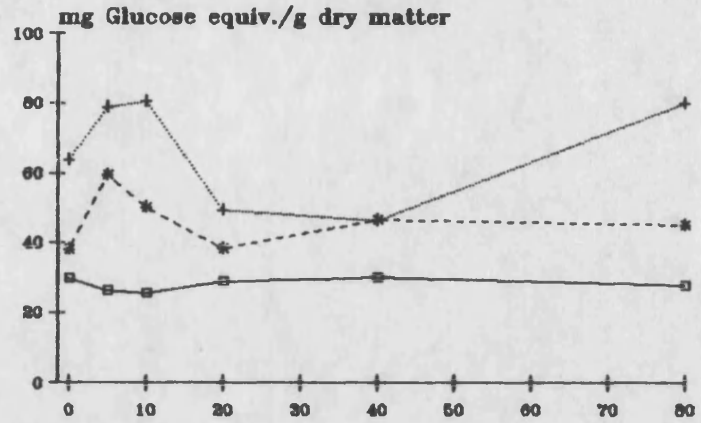


Reducing sugars were higher in the expanding and hard leaves of plants treated with the lowest PBZ rates compared to untreated plants. Root reducing sugars were not influenced by application of PBZ (Fig. 5.7a). Applications of higher PBZ rates did not affect reducing sugar levels in leaves and roots of treated plants.

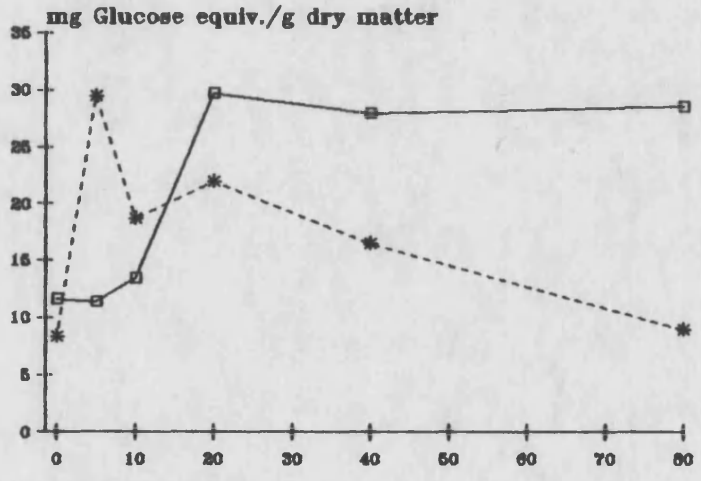
Non-reducing sugars were not determined in expanding leaves. Plants treated with the lowest PBZ rate had the highest leaf non-reducing sugar levels. Higher rates of PBZ did not affect leaf non-reducing sugars. Root non-reducing sugars were lowest in untreated plants and those receiving lower rates of PBZ but dramatically increased in higher PBZ plants (Fig. 5.7b).

Starch concentration was noticeably increased in the expanding leaves of plants receiving low rates of PBZ compared to untreated plants and plants receiving higher rates of PBZ (Fig. 5.7c). Levels of starch in hard leaves and roots were not affected by PBZ application.

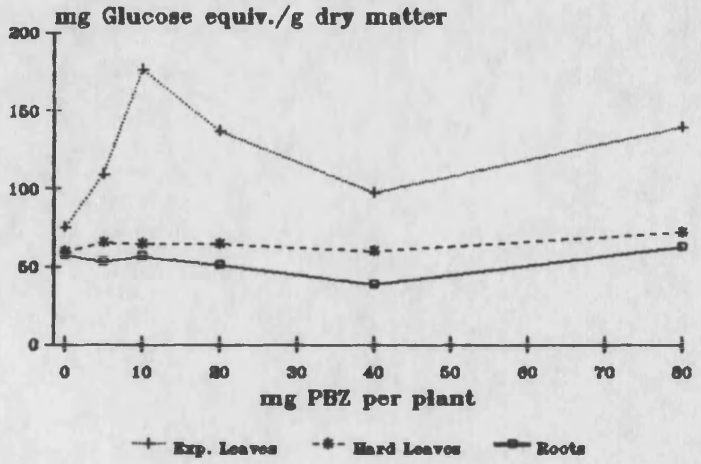
**Fig. 5.7 Effects of PBZ on Levels of (a) Reducing (b) Non-reducing sugars and (c) Starch in leaves and roots.**




(a) Reducing sugars




(b) Non-reducing sugars




(c Starch

## 5.4. Discussion.

Applications of high rates of paclobutrazol resulted in reduced stem extension growth primarily as a result of the strong inhibition of internode elongation. While extension growth was stimulated at low rates of PBZ applied to three and five month old seedlings, higher PBZ rates inhibited extension growth at the end of a three month growing period. The stimulation or inhibition of extension growth were closely positively related with the changes in internode lengths at various PBZ rates. Most research on PBZ has shown reductions in elongation growth depending on application rate (Wood, 1984; Snowball, Halligan and Mullins, 1988; Williams, Biscay and Smith, 1989; Wolstenholme, Whiley and Saranah, 1990), the reductions in growth being largely attributed to effects on internode elongation.

However, there have been very few reports linking PBZ with stimulation of growth. Liyembani and Taylor (1989) reported stimulation of growth in peach seedlings with applications of 7.5 mg PBZ, while higher rates inhibited growth. Reasons for this are not clear. Casper and Taylor (1989) argued that growth stimulation following application of PBZ mostly involved the development of short shoots or the elongation of long shoots which were beginning to slow in growth. In other words, the effects of low rates of PBZ were dependent on stage of growth. Steffens and Wang (1984) noted that, in general PBZ has the greatest retardant effects on tissues which are rapidly growing and developing at the time of treatment or shortly after. It is, therefore, possible that the stimulation of growth in seedlings at six and eight months of age following PBZ application at 3 and 5 months respectively, could be due to sensitivity to PBZ depending on stage of growth at application. Unfortunately the stage of plant growth at time of PBZ application was not documented so this theory can not be examined further in this case.

Another possible explanation for the stimulation of growth is that the PBZ rates were too low to continuously inhibit GA biosynthesis in a strong flush of growth. As a

result, there was compensatory growth which is reflected in the elongated internodes. At higher PBZ rates there is a PBZ dose related reduction in extension growth. This is very important as very high rates of PBZ may eventually lead to a complete inhibition of shoot extension and leaf formation. It has been reported (Wood, 1988) that a loss of nut production can occur in mature pecan trees three to four years following PBZ application, attributed to reduced tree assimilate levels due to decreased shoot growth and a decline in leaf area.

The effects of PBZ application on leaf area were mostly related to an increase in the number of leaves rather than leaf size. The increases in total leaf area at low PBZ rates were due to an increase in the number of leaves following growth stimulation. These findings are similar to those reported by Steffens, Byun and Wang (1985) and confirms that PBZ exerts most of its effects on stem extension growth and that an optimum level of leaf area is still maintained. It is also possible that slight reductions in leaf area at higher PBZ rates could be as a result of the inhibition of cell multiplication in the developing leaf. PBZ has been reported to inhibit cell multiplication in cell suspensions of Paul's Scarlet Rose (Bayliss, 1984), although cell volume and dry weight increase were not prevented and inhibited cultures therefore produced greatly enlarged cells.

It is apparent that very high rates of PBZ may completely inhibit stem extension and affect leaf production. At the highest PBZ rates growth was markedly reduced and shoots developed thick stems and due to reduced internodes. The shoots appeared rosetted. This is likely to result in mutual shading of leaves and the photosynthetic efficiency of the leaves may be affected. At optimum PBZ rates, the advantage may be that since leaf areas are not affected but extension growth is inhibited, assimilates produced can be utilised elsewhere, for instance in reproductive growth in mature trees.

The bulk of the seedling dry matter was allocated to shoots. Macadamia seedlings generally allocate relatively little dry matter to the root system unlike crops such as pecan (Carya illinoensis) where the seedlings allocate more dry matter to the root than to the shoot (Wood, 1988). Treatment with PBZ resulted in further reductions in the dry matter of the macadamia root system. However, this does not necessarily imply poor root production as it could be the result of the production of a higher proportion of fibrous as opposed to thick roots. Indeed Wang, Byun and Steffens (1985) reported increased numbers of short roots with enlarged diameter following PBZ application to apple seedlings. They found that root growth could be either increased or decreased depending on PBZ dose duration of treatment and method of application.

From the work reported in Chapter 3 it was noted that macadamia leaves developed very rapidly which might mean that they change from acting as a heavy sink to a source within a short period of time. The regulation of growth of such strong sinks should, therefore, enable changes in assimilate distribution. The use of the regulator PBZ had an effect on carbohydrate levels in the seedlings. Plants receiving the lowest PBZ rates (5 and 10mg) had higher levels of reducing sugars and starch compared to untreated controls and those at higher PBZ rates. In addition, TNSC levels in expanding and hard leaves were higher in the lowest PBZ plants.

Some research has been conducted on the effects of PBZ on carbohydrate distribution in plants. Steffens et. al. (1986) reported that treatment of apple seedlings with PBZ shifted assimilate partitioning in apples from leaves to roots. Roots from treated plants accumulated the most carbohydrates. However, in the case of macadamia seedlings here, roots had the least TNSC levels. The differences are probably due to the fact that in the apple seedlings reported above, 70% of the root carbohydrate was from the taproot, with fibrous roots contributing only 30%. Since the macadamia does not possess a taproot and generally develops a rather small root system, it clearly

plays no significant carbohydrate storage role, most of the reserves being kept in the leaves.

Research on apple seedlings by Wang, Byun and Steffens (1985) has indicated that substantial accumulation of total carbohydrates occurs in old leaves following PBZ application. This is in agreement with the findings here except that in macadamia, the expanding leaves had more carbohydrates at all levels of PBZ compared to hard leaves. In the case of apples (Wang et. al. (1985), new leaves had lower carbohydrates than old leaves. There is no indication as to whether the term "new leaves" used by these authors referred to leaves formed following PBZ application, newly developing leaves or indeed whether they were hardened or expanding.

In macadamia, expanding (developing) leaves are a very active sink (Allan, 1972), hence assimilates are translocated here for plant building processes. The stimulation of growth at low PBZ rates coincides with increases in sugar levels at the same rates. Stimulated growth was mostly a result of flush growth in these plants, whereas plants receiving higher PBZ rates had very little flush growth hence no growth stimulation at all. It is not clear whether the stimulation in extension growth at the low PBZ rates was a consequence of the higher sugar levels in the tissues or indeed vice versa.

Of the component carbohydrates in the macadamia seedlings, starch was in the highest amount while non-reducing sugar levels were very low. Both starch and reducing sugars were higher at the lowest PBZ rates compared to untreated controls and higher PBZ plants. This is in agreement with findings reported by Wood (1984) on pecan seedlings. He reported increases in starch and reducing sugar accumulation at PBZ rates of 0.5 and 1mg/plant. As in the pecan seedlings, higher rates of PBZ resulted in reduced accumulation of sugars in the macadamia seedlings. In both pecan (Wood, 1984) and apple (Wang et. al. (1985) seedlings, the taproot had the highest total sugars at all PBZ rates. The taproot, therefore, appears to be a very important

storage structure for carbohydrates in seedlings. In macadamia seedlings, however, most sugars were found in the leaves and very little in its mostly fibrous root system. The lack of the taproot in these seedlings may, therefore, be the main reason for the low allocation of dry matter to the root system in this plant.

It is quite possible that high levels of storage sugars are available in the wood or bark of the seedlings, but as these were not determined (due largely to the small size of the bark and problems with grinding whole stem dry matter) it is not possible to evaluate this and its importance in shoot growth. Stephenson, Gallagher and Rasmussen (1989) have reported high levels of storage sugars (starch) in the wood and bark of mature macadamia trees. Assuming this was the case with the seedlings as well, then the total sugars in the shoots will be much higher than those of the roots. In flushing seedlings, therefore, the actively growing points and their expanding leaves will act as a stronger sink than the root system and this may result in the accumulation of higher sugars in the expanding leaves. The roots, on the other hand, are then continually outpaced by the shoot.

A more detailed study of carbohydrate partitioning between the root and shoot system in the seedlings would repay further investigation. Although this was examined systematically, it seems possible that the roots may also grow in flushes, since active white roots were seen only at certain times while at other times the root system looked brown and rather inactive even when the shoots were still growing. If the root system does grow cyclically, it would then be interesting to determine the relationship between root and shoot flushes and how these might be related to carbohydrate distribution.

CHAPTER 6.

# EFFECT OF PBZ ON THE GROWTH, SUGAR AND STARCH CONTENT AND YIELD CHARACTERISTICS OF BEARING MACADAMIA TREES IN MALAWI.

## 6.1. INTRODUCTION

Macadamia have been described as an overstory rainforest spp. (Trochoulias, 1990) and as an upper canopy species (Lloyd, Trochoulias and Ensbey, 1990) with respect to their potential size under suitable conditions. Macadamia trees grow rapidly and form huge canopies, commonly exceeding 20m high and 15m wide on mature trees. As a result they have to be planted at relatively low plant populations and are prone to wind damage. Apart from the inherent competition between vegetative and reproductive growth, which often results in low productivity, the management of such large trees has proved rather difficult.

Several spacings have been recommended for macadamia under Australian conditions. Trochoulias and Burnside (1988) stipulated a 10 x 5m spacing as being adequate for most clones with the removal of each second tree after 12 years to give a 10 x 10m final spacing. In Malawi, where trees grow more vegetatively than in Australia, a 10m X 10m spacing is widely used accompanied by interplants of coffee and other crops to fully utilise the space between macadamia trees in the early years.

Research with high density macadamia plantings without the use of dwarfing rootstocks or chemical agents has not been very successful. Newett (1988) reported on densities of up to 895 trees per hectare where trees became crowded after five years and yields depressed by the sixth year on some clones. Benefits of close planting included creation of a microclimate which reduced high temperature chlorosis compared to wide spaced trees. Trochoulias and Burnside (1988) assessed

several high density plantings in Australia, one of the oldest being at 5 x 3m and trained to a wire trellis using a modified palmette system. Yields of one high density clone at five years were comparable to ten year old trees spaced at 10 x 5m. However, the system eventually became unmanageable even with mechanical pruning.

Pruning is laborious and there is strong evidence that frequent or heavy pruning is detrimental to early cropping in macadamia (Trochoulias, 1983). Heavy pruning also causes a reduction of tree girth and size, which delays cropping by at least a year. In the first season following pruning, yields may be reduced if significant bearing wood is removed. The tree may, however, have sufficient reserves and the capability of setting and carrying a crop on the remaining fruiting wood. In hedge-row macadamia systems in Australia, topping-up has been practised (Cull, Stephenson and Winks, 1983). This involves hedging the tree at a certain height. Topping-up, although removing yield potential higher up, may foster continuing production on the lower parts of the canopy. This lower area of the canopy is easier and cheaper to reach for pest control.

Dense canopies also have implications in pest control strategies. Spray application has constraints related to height and penetration throughout the canopy. The maximum effective penetration by existing air blast sprayers without towers is in the order of 7m. It must be recognised that insect pests such as macadamia nut borer (Cryptophlebia ambrodelta), fruit spotting bug (Amblyopelta nitida) and scale insects can congregate and move from unsprayed sections of the trees. Therefore, not only are unsprayed sections possibly unproductive, but they threaten other sections of the tree and may add significantly to lowering the average nut quality on the tree.

Shading also increases in dense canopy trees as plants grow closer together and higher. It is, therefore, important to know the light threshold level inside the tree canopy below which yield and quality are adversely affected. Apart from being

converted into chemical energy through photosynthesis, light also directly regulates growth, development and the form of plants, including vegetative and reproductive growth, through photomorphogenesis and photoperiodism. Cull (1980) observed that yield per unit volume of tree canopy declined as trees grew older and the zone of the canopy producing nuts tended to move closer to the canopy surface where there was more light. Carbohydrate production was related to the proportion of leaf area exposed to medium to high light intensity.

Crucially important for fruit set in tree crops is the need to balance competition between the indeterminate and determinate growth of the vegetative and reproductive meristems respectively. Thus the most fundamental step a horticulturist can take to amplify fruitlet competitive ability is to reduce tree vigour (Crannel, 1985). Excessive vegetative growth results in dense canopies with little solar radiation reaching interior leaves and the fruit, a microclimate which has been associated with poor fruit quality (Priestley, 1962).

Current flushes greatly influence vegetative growth in macadamia and probably determine the competitive interactions between vegetative and reproductive growth. Macadamia growth is achieved through a series of minor and major growth flushes. The flowers are the first part of the renewal shoot to expand in spring, but subsequently a vegetative shoot usually elongates from the terminal vegetative bud. Thus vegetative and reproductive spring growth do not coincide precisely, however there is usually a considerable degree of overlap and direct competition for resources. Competition between vegetative and reproductive growth is believed to be at least partly responsible for the poor fruit set characteristic of macadamia. In heavy cropping seasons, a final opportunity for adjustment of the crop load is provided by competition between rapidly growing nuts and the summer growth flush. In most cases there is a substantial premature nut drop which has also been associated with environmental factors.

Stephenson and Cull (1986b) confirmed the dominant controlling effect of temperature on vegetative flushing. They found that major flushes in south east Queensland were restricted to times of the year when temperatures were relatively mild. The major summer flush occurred either towards the end of a period of above threshold temperature or after the more extreme parts of it. Presumably when high temperatures inhibit flushing the tree reserves continue to accumulate. With the onset of milder days in February and March, the accumulated reserves would then be available to promote vigorous flushing. The pattern of vegetative and reproductive growth and their seasonality for Southern Malawi conditions have been detailed in Fig. 1.1 of Chapter 1.1.8.

Of the two major flushes, the summer flush is often more vigorous than in the spring. Stephenson and Gallagher (1983) reported that the average amount of growth per terminal was about ten times greater in summer than during spring. In addition, a greater proportion of terminals undergo flushing in the summer period. In a multiple regression model developed for trees growing under Australian conditions by Stephenson and Cull (1986b) flushing status was found to account for 8.2% of the variation in yield, especially the absence of flushes between October and January, when nut set, rapid nut growth and oil accumulation were occurring. In contrast, flushing activity during August/September was beneficial to yield possibly by providing actively photosynthesising foliage to support nut growth and oil accumulation.

Growth flushes have also been reported to influence, and be influenced by, nutrient status in the plant. In the absence of major reproductive or vegetative activity in the tree N, P, and K continue to accumulate. In Australia, depletion of these elements in spring and late summer has been primarily associated with increased demand by vegetative flushes although flowering, nut set and nut growth apparently also

contribute to the large decline in spring (Stephenson et al., 1986b). It has been reported that in avocados the finely balanced vegetative/reproductive competition is easily tipped in favour of vegetative growth by over-stimulative conditions such as nitrogen application (Wolstenholme et. al., 1990).

It appears that most of the problems associated with macadamia productivity would be overcome if a more manageable tree in terms of size and structure was developed. This would require the regulation of tree growth in order to maintain a smaller tree size also to ensure productivity. This could also result in higher plant populations, lower growing costs per tree, and higher yields per unit area. One method for controlling the vigour of the growth flushes is to manipulate the shoot physically by tipping or girdling and thus temporarily reducing vegetative sink strength. This temporary respite from competition can allow fruit setting (Williams, 1980). However, although shoot tip pinching and girdling are useful methods for studying the competitive interactions between fruit set and flushing events in macadamias, they offer little scope for increased productivity in orchards because of their high labour costs.

Chemical growth retardants such as PBZ have the practical advantage of ease of application and their ability to reduce shoot vigour at times favourable to fruit set has already been reported in other tree crops (Quinlan, 1980; Lever, Shearing and Batch, 1982; Shearing and Jones, 1986; Webster and Quinlan, 1986; Curry, 1988). There is therefore the possibility that the use of growth retardants such as PBZ on mature macadamia trees could result in the development of more limbs, a compact and hence structurally stronger tree which is less prone to wind damage. Effects on the external tree shape would also improve structural stability. The long term objective would be to achieve a physiological balance within the tree in support of high nut yields together with sufficient vegetative growth to provide potential fruiting sites for sustained future production.

There has been little use of growth regulating chemicals on macadamia but PBZ has already been shown to reduce growth on seedling macadamias in this study (Chapter 5) and Stephenson et. al. (1989) have reported the use of PBZ to manipulate growth in mature macadamia trees in Australia. Their work showed that PBZ sprays were effective in temporarily depressing vegetative flushing. In treated trees, storage carbohydrate levels declined with the onset of spring flush and flowering and remained low until after rapid nut growth had ceased. Due to poor timing of PBZ application, treated trees had low inflorescence density and poor early anthesis. Nakata, Long and Suehisa (1973) reported a 10% increase in yield following treatment of macadamia trees in Hawaii with daminozide, another retardant. It is not clear whether the effect was related to greater initial fruit set, a reduction in premature nut drop, a modification in growth parameters or a combination of the three factors. There have been no published reports of the use of growth retardants on macadamia in Southern Africa.

Some work has also been conducted on the use of growth regulators to manipulate fruit abscission in macadamia. Nagao and Sakai (1985) reported increased of fruit removal force (FRF) in macadamia explants following treatment with ethephon. Application of silver nitrate inhibited the reduction in FRF as did NAA, while gibberellic acid and benzyladenine had no effect. Kadman and Ben-Tal (1983) found that sprays of ethephon, applied to mature trees of Beaumont macadamia trees at the beginning of the harvest season [redacted], resulted in more than 90% nut drop without causing damage to the foliage. Nagao and Sakai (1988) have shown that macadamia fruits display a differential sensitivity to ethephon during development. Fruit abscission can be stimulated by ethephon during the early stages of growth, followed by a second period of increased sensitivity as fruits mature. All this indicates that macadamia trees respond to chemical treatments, therefore their growth may have the potential to be controlled with judicious use of such chemicals.

## 6.2. AIMS OF STUDY

The study was aimed at determining the practical effects of paclobutrazol on the growth and productivity of young bearing macadamia trees in Malawi. Special attention was paid to three aspects in the study: Effects on vegetative growth; yield and quality characteristics; leaf carbohydrate levels, and relationships between all three aspects.

## 6.3. MATERIALS AND METHODS

A trial was established, using 5 year old macadamia trees of clone 246 (Keauhou) which were just beginning to bear (Plate 6.1), at Bvumbwe Agriculture Research Station (16$^{o}$S latitude and 1200m altitude) in Southern Malawi. A randomised complete block design incorporating three chemical treatments and an untreated control in two-tree plots with 4 replications was used. The growth retardant, 'Cultar' (23% W/W paclobutrazol, ICI Agrochemicals, UK Ltd), was applied at 2, 3 and 4g a.i. paclobutrazol per tree. Cultar was applied in 1 litre of water to the base of the tree at the end of February 1989 and 1990.

Following the summer growth flush in December, five shoots with growth flushes were tagged on each tree and the resulting growth measured in mid-February after the flushes had fully developed. Trunk girth was measured 30cm above the graft union before PBZ application in the first year and also 12 months later. Canopy cover was measured as the diameter of the dripline across both the North-South and East-West axes in the first season of growth after PBZ application.

Mature nuts were picked from the ground beginning in December. At the end of February the trees were stripped of all remaining nuts. Harvested nuts were dehusked and dried in the shade for a week to bring the moisture levels down to 10%, and the nuts weighed to give in-shell nut yield. A sample of 100 nuts was dried in the oven at 105$^{0}$C for 72 hours for quality assessment. Kernel recovery was assessed as percentage of kernels to nuts by weight. Kernels were soaked in water and all floaters taken as Grade 1.

Leaf samples for carbohydrate determination were collected in January 1991. Two flushing shoots were sampled, one from the east and another from the south side of each tree. These sides were chosen because they contained the most flushes. Leaf samples were dried in an oven at 95$^{0}$C for one hour and subsequently 70$^{0}$C for four

hours. Dried leaves were ground using a plant grinder (Arthur H Thomas Scientific Apparatus, Philadelphia PA USA) fitted with a 1mm sieve. Samples were shipped to Bath University for carbohydrate analysis.

All data on growth and quality were subjected to a two-way ANOVA analysis in Minitab (Appendix 2F).

Plate 6.1 Size of trees at time of first application of PBZ in February 1989. Note that the summer flush is just coming to an end, indicated by the light green new shoots at the top and sides of the tree.

## 6.4. RESULTS

The application of PBZ significantly affected shoot growth of trees in the two years following application (Table 6.1, Figs. 6.1a and b). In the first year of application (1990), there were significant differences in shoot growth (P = 0.001) and internode length (P=0.05) between paclobutrazol treatments. Applications of lowest rate of PBZ (2g) resulted in production of shoots 30% longer than those on untreated trees. Increases in PBZ rates to 3 and 4g a.i. resulted in flushes 6 and 20% shorter than those in untreated trees for the respective rates.

In the second year of application (1991) there were also significant differences in flush length (P = 0.001) and internode length (P = 0.05) between PBZ treatments. However, the small apparent growth stimulation at the lowest PBZ rate was not significant unlike the first year of application. Generally the second year flushes were shorter than the previous year's in all treated and untreated trees. Internode lengths reflect similar patterns as flush growth. The data suggest that increases or reductions in flush length were as a result of changes in internode length (Figs. 6.1a and b) as the patterns are very similar. Plates 6.2 and 6.3 shows flushing shoots from trees treated with low and high rates of PBZ.

PBZ treatments had no significant effects on growth parameters such as trunk girth and canopy cover in the first year of application (Table 6.1). Similarly PBZ treatments had no significant effect on in-shell nut yield in the first year, although trees receiving 4g a.i PBZ had 31% higher yields than untreated trees. In the second year, however, there were significant differences in yield between PBZ treatments (P=0.05). Trees treated with the lowest PBZ rate had the lowest nut yield, while untreated trees had similar yields to those treated with higher PBZ rates.

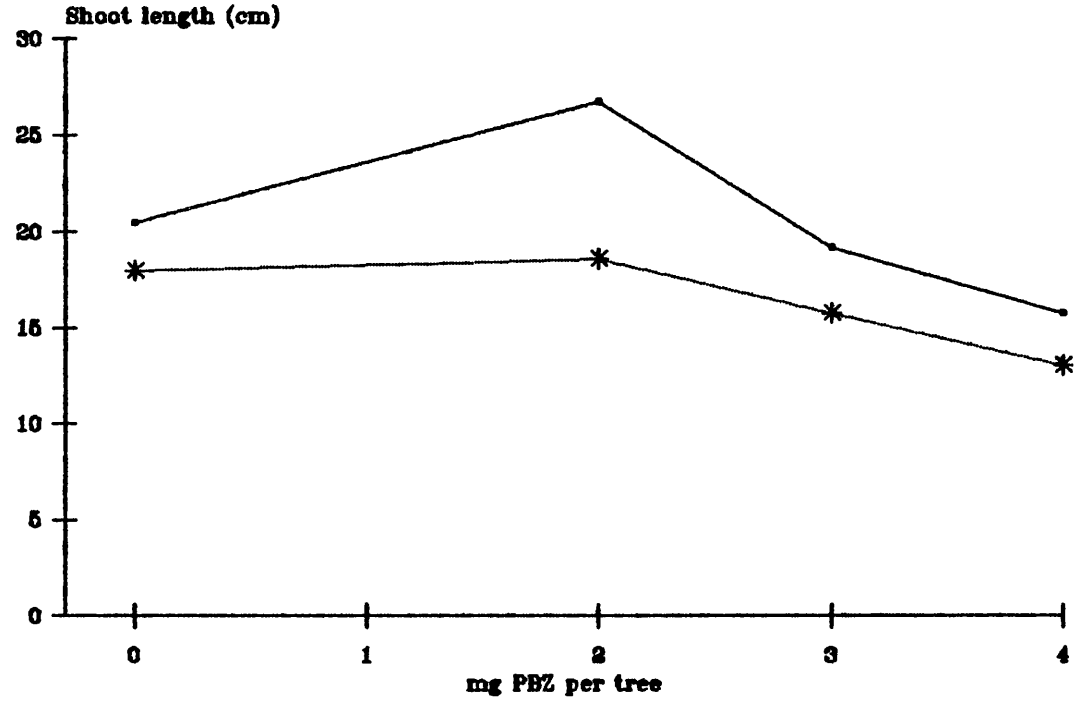
PBZ had no significant effect on kernel recovery levels in both years. In the first year, however, nuts from trees with 4g PBZ had 10% higher kernel recovery than those

Table 6.1 Growth, yield and quality characteristics of 6 year old bearing macadamia trees following treatment with Paclobutrazol.

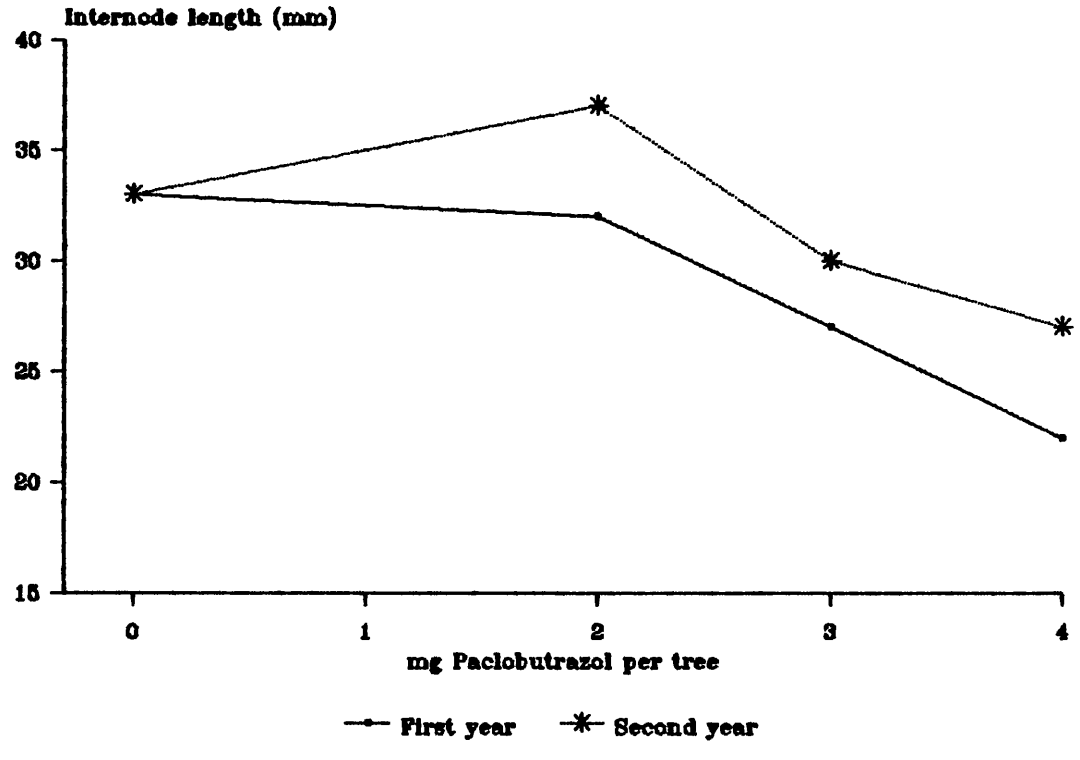
| | | g. Paclobutrazol per tree | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Control | 2 | 3 | 4 | S.E.D | Sign. | LSD(5%) |
| Flush length (cm) | 1990 | $20.43^{b}$ | $26.71^{c}$ | $19.16^{ab}$ | $15.74^{a}$ | 1.98 | *** | 3.88 |
| | 1991 | $17.95^{c}$ | $18.58^{c}$ | $15.73^{b}$ | $13.01^{a}$ | 0.71 | *** | 1.39 |
| Internode length | 1990 | $33.00^{ab}$ | $37.00^{bc}$ | $30.00^{ab}$ | $27.00^{a}$ | 2.50 | * | |
| (mm) | 1991 | 33.00 | $32.00^{bc}$ | $27.00^{ab}$ | $22.00^{a}$* | 0.16 | | |
| Trunk girth (cm) | 1990 | 34.00 | 36.75 | 37.13 | 36.75 | 2.06 | NS | |
| Canopy cover (m) | 1990 | 3.58 | 3.63 | 3.66 | 3.77 | 0.32 | NS | |
| Nut yield | 1990 | 474.9 | 489.9 | 480.5 | 622.8 | 75.36 | NS | |
| (g/tree) | 1991 | $788.7^{b}$ | $707.5^{a}$ | $736.3^{ab}$ | $765.0^{b}$ | 24.21 | * | 51.32 |
| Kernel recovery | 1990 | 24.59 | 20.38 | 26.29 | 27.25 | 3.22 | NS | |
| (%) | 1991 | 28.75 | 28.23 | 28.08 | 28.28 | 1.55 | NS | |
| % Grade 1 | 1990 | 65.54 | 59.91 | 69.87 | 77.34 | 10.10 | NS | |
| | 1991 | $87.64^{bc}$ | $76.20^{a}$ | $78.79^{a}b$ | $91.81^{bc}$ | 4.59 | * | 9.73 |

Means followed by the same letter in each row are not significantly different at P=0.05

**Fig.6.1 (a) shoot extension (cm) and (b) internode length (mm) on a summer flush in bearing trees treated with PBZ**




(a) Shoot extension




(b) Internode length (mm)


Plate 6.2 A flush shoot from a tree treated with 2mg PBZ. Note the relatively long internodes on the new flush.


Plate 6.3 A flush shoot from a tree treated with 4mg PBZ. Note the relatively short internodes and the increased number of leaves on the new shoot.

from control trees. The lowest recoveries were obtained on nuts from 2g PBZ. Similarly, nuts from trees receiving high rates of PBZ had 19% more grade 1 kernels than those from untreated trees.

In the second year kernel recovery was more or less the same for all treatments. However, PBZ had a significant effect (P=0.05) on % grade 1 kernel. Nuts from trees treated with 4g PBZ had 4% and 20% more grade 1 kernels than those from untreated controls and lower PBZ rates respectively. In both years, the lowest PBZ rate resulted in lower grade 1 kernels while higher PBZ levels resulted in increased grade 1 kernels. The increase in kernel recovery was only evident in the first year. Also worth noting is a general improvement in nut quality with tree age, as % grade 1 kernels were higher in the second than in the first year.

The data suggest some relationship between vegetative growth and reproductive growth in terms of kernel recovery and quality. At the lowest PBZ application rate, where flush growth was stimulated, the kernel recovery and grade 1 were relatively low. However, higher PBZ levels resulted in reductions in flush length and higher kernel recovery and grade 1 levels. Significant negative relations were obtained between flush length and kernel recovery ($P = 0.05$, $R^2 = 96.7\%$). There was also an apparent, though not significant, negative relation between flush length and grade 1 kernels ($R^2 = 88.7\%$).

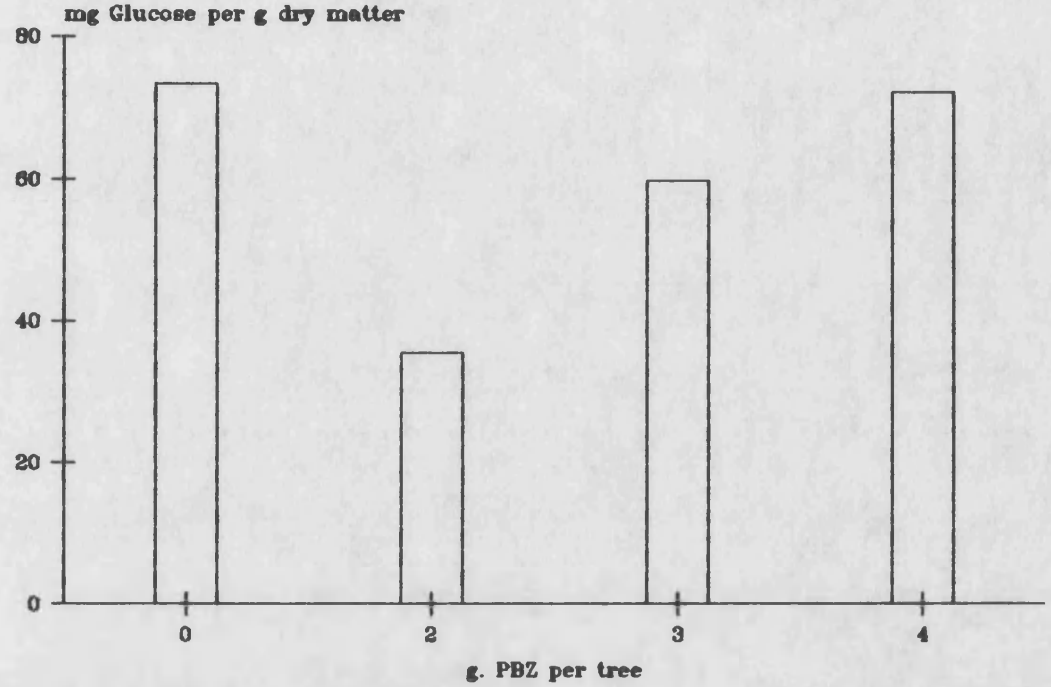
The application of PBZ had significant effects ($P = 0.001$) on reducing sugar and starch levels in leaves (Table 6.2) although the level of non-reducing sugars was not affected significantly. Leaf samples from trees receiving 2 and 3g PBZ had, respectively, 51% and 18% lower reducing sugars than controls while those from trees receiving 4g had similar levels as controls (Fig. 6.2a). The trend of starch levels was rather different. All PBZ-treated trees had higher levels of starch than untreated

Table 6.2. Leaf Carbohydrate levels in 6 year old macadamia trees following treatment with paclobutrazol for two seasons. Leaf samples were collected from a flushing shoot at the end of the major summer flush in 1991. Values are expressed as mg. glucose equivalents per g. dry matter.

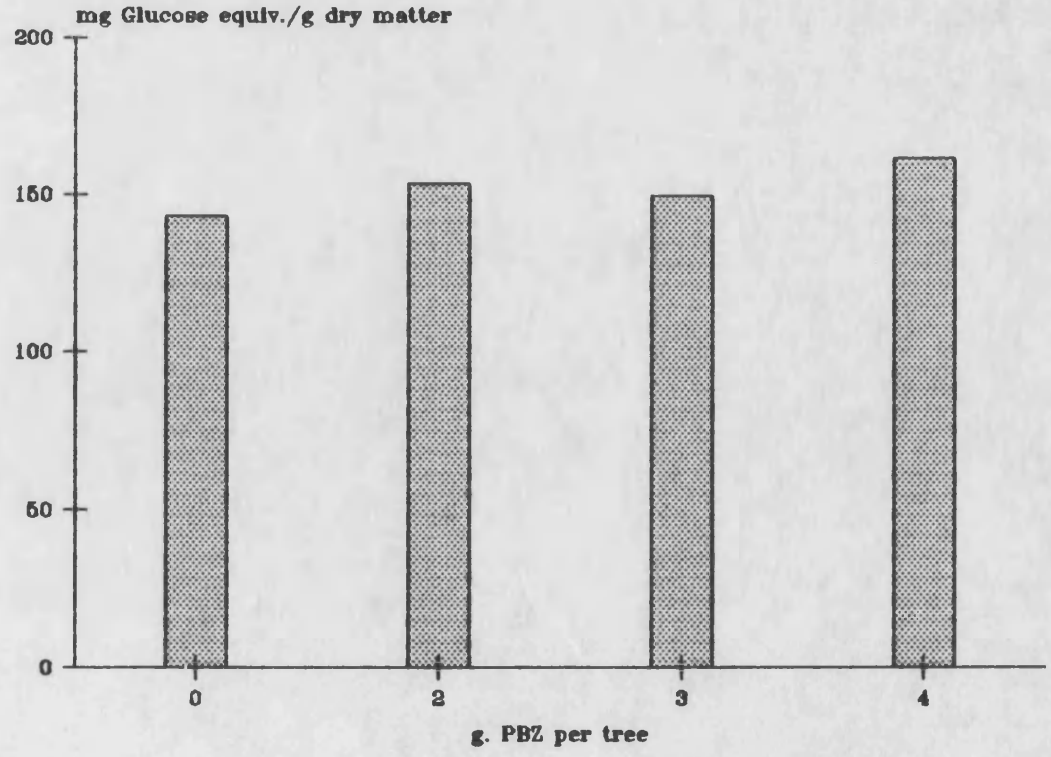
| g. PBZ per tree | Reducing sugars | Non-reducing | Starch | TNSC's |
|---|---|---|---|---|
| Control | 73.32[c] | 30.57 | 143.1[a] | 246.9[b] |
| 2 | 35.44[a] | 14.06 | 153.4[c] | 202.9[a] |
| 3 | 59.66[b] | 28.35 | 149.6[b] | 237.6[b] |
| 4 | 72.16[c] | 23.79 | 161.3[d] | 257.3[b] |
| SED | 0.60 | 4.68 | 0.49 | 7.8 |
| Sign. | *** | NS | *** | *** |
| LSD 5% | 1.91 | | 1.56 | 24.80 |

[1]Means followed by the same letter in each column are not significantly different at P=0.05.

**Fig.6.2 (a) Reducing sugar and (b)starch levels in leaves of 6 year old trees following treatment with PBZ for 2 years**




(a) Reducing sugars

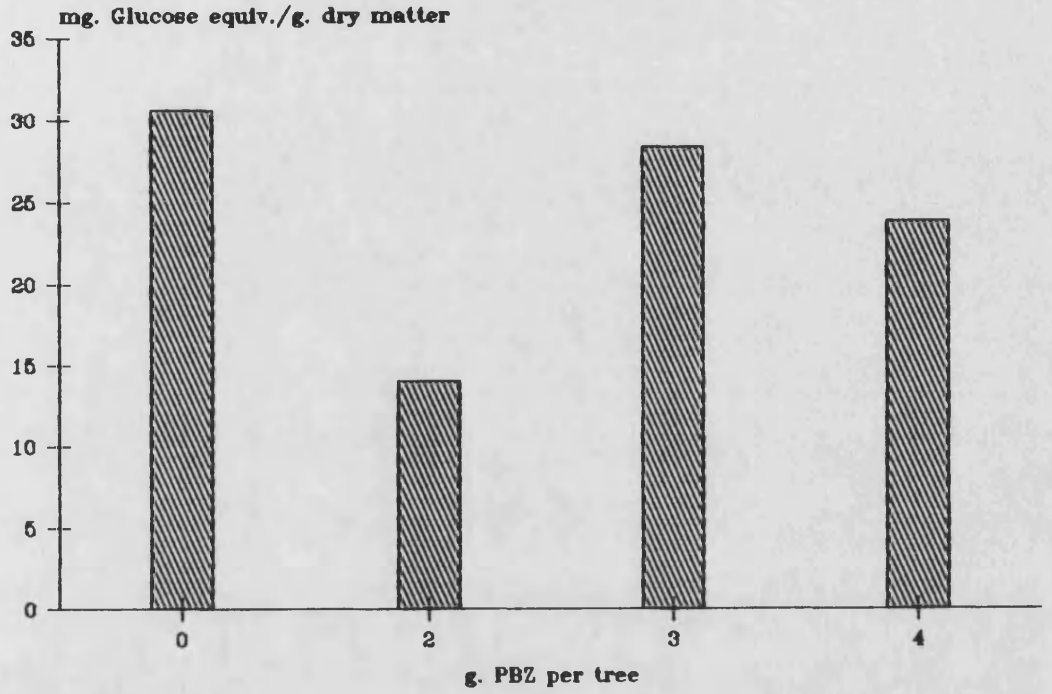



(b) Starch

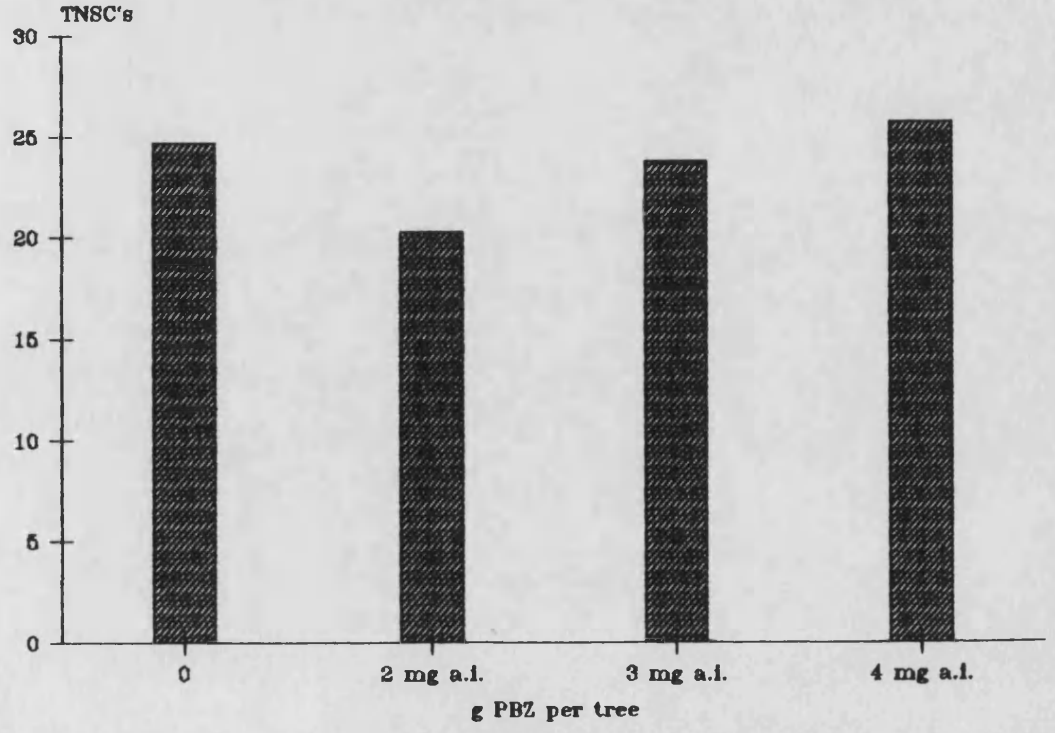
Although PBZ had no significant effect on non-reducing sugar levels the general trend shows that all treated trees had lower levels than untreated trees (Fig. 6.3a). The lowest levels were in trees receiving 2mg a.i., with 54% lower than untreated trees. Leaves from trees with higher PBZ rates had non-reducing sugars only slightly lower than control trees.

Trees treated with 4g PBZ had the highest leaf TNSC (4% higher than control), while those treated with 2g had lowest TNSC levels (17% lower than untreated trees) (Fig. 6.3b).

**Fig. 6.3 (a) Non-reducing sugar and (b) TNSC levels in leaves of 6 year old trees treated with PBZ for 2 years.**




(a) Non-reducing sugars




(b) TNSC's – expressed as mg Glucose equivalents per g dry matter

## 6.5. Discussion

Significant reductions in shoot growth and internode length were obtained following application of high rates of PBZ in the two years. However, in the first year, growth was stimulated in trees receiving 2g PBZ, resulting in flushes 30% or more longer than those of untreated controls and trees receiving more PBZ. In the second year of application the growth stimulation at the same rate was not as significant. As in the macadamia seedlings, increases and reductions in extension growth were directly related to internode length, further confirming effect of PBZ on internode elongation.

Reductions in shoot growth and internode lengths following PBZ application have been reported in a variety of tree crops including apples (Stinchcombe et. al., 1984), peach (Casper and Taylor, 1989), pecans (Wood, 1988). Lever (1986) noted that the most marked effect of PBZ was a dose related reduction in internode length on terminal and lateral shoots. This was the case here although application of 2g PBZ resulted in longer internodes and increased growth. The short internodes create the opportunity and potential for more flower racemes as more nodes are developed.

Stimulation of growth following the application of a low dose of PBZ was found in our work with macadamia seedlings (Chapter 5). It has also been reported on one year old peach seedlings by Liyembani and Taylor (1989). However, most studies on mature trees indicate progressive reductions in shoot length with PBZ application (Wood, 1984; Snowball et. al., 1988; Williams et. al., 1989). Reductions of up to 40% in spring flush shoot length have been achieved following PBZ application to six year old avocado trees. PBZ was applied at rates of 2.5-5g a.i per tree and by this means the phenological growth cycle was manipulated at the critical stages of fruit set and summer fruit drop Wolstenholme et. al., 1990). Most of these reporters attributed the effect to reductions in internode length.

The effect of PBZ on growth has been associated with its effect on the restriction of cell division (Bayliss, 1984; Dalziel and Lawrence, 1984). The stimulation of growth at the lowest PBZ rates was perhaps a result of the plant overcoming the retardant effects and hence producing compensatory growth which was more vigorous than the original flush. In the case of the macadamia, it would be instructive to determine if the flush in PBZ-treated trees occurred at the same time as in untreated trees and establish if there is any delayed flushing in the former as the plants try to overcome the inhibitory effect of the chemical.

Application of PBZ had no significant effects on trunk growth and canopy spread. Trunk girth and canopy spread are presumably the result of the accumulation of several years annual increment in trunk diameter and branch formation. It is unlikely that the effect of PBZ in a single year, or even over two years, on this increment would have any appreciable effect. Casper and Taylor (1989) have reported reductions in trunk cross-sectional area of young peach trees following PBZ application. Apart from stabilising the tree, the macadamia trunk has been reported to hold high deposits of storage carbohydrates (Stephenson et. al., 1989). Reduced vegetative growth following PBZ application may lead to the development of a larger trunk which could then provide an important source of carbohydrates for reproductive growth.

PBZ had very little effect on yield and quality factors. The high yields in trees receiving 4g PBZ in 1990 were perhaps a result of precociousness. There was a general trend of increasing yields with age from 1990 to 1991. This is also reflected in kernel recovery levels. Research on large pecan trees by Wood (1988) has shown increases in in-shell nut yield in the second year after PBZ-soil application. However, in PBZ-injected trees, in-shell nut yield was reduced in the third and fourth years, presumably due to decreased leaf area resulting from the greater inhibition of shoot growth. This trend was not repeated in the following year.

It is interesting to note that as in the seedling macadamias, the non-reducing sugar levels have generally been lower than reducing sugars while starch levels have been very high. Both reducing and non-reducing sugars were much lower in leaves from trees with the lowest PBZ. This is particularly interesting as it coincides with the stimulation in growth of flushes at this PBZ rate. The 30% stimulation in growth coincides with 51% and 54% reductions in reducing and non-reducing sugars respectively. Since leaf samples were collected at the end of the vigorous summer flush it can be speculated that the soluble sugars in these trees were depleted due to increased demand as a result of the growth stimulation. Leaves from trees receiving the highest PBZ levels do not show any differences in terms of soluble sugars compared to untreated trees.

Trees treated with the highest PBZ levels had leaf starch levels 12% higher than untreated trees. This shows that these trees were able to store carbohydrates which could be used for some processes other than vegetative growth. The low starch levels in leaves from control and low PBZ trees was probably as a result of dissolution of reserves and retranslocation of the solutes to active shoots.

It is well known that carbohydrates are stored by woody plants (Zimmerman 1971) and utilised during periods of demand during new growth in spring (Priestley, 1962). However, the importance of the storage carbohydrates in subtropical, evergreen fruit trees in which current photosynthesis makes continuous contribution to the trees' assimilate requirements throughout the year is not so well known. Depletion of such reserves during flowering, shoot growth and nut development in macadamias (Cormack and Bate, 1976) and avocado (Scholefield et. al., 1985) suggests that those processes may depend on storage carbohydrates. In the present study, the low starch levels in low PBZ and control trees coupled with increased extension growth in those

treatments compared to high PBZ trees could be considered to confirm that storage carbohydrates were used to support the increased growth.

Trees treated with the highest PBZ levels had more leaf total non-structural carbohydrates (TNSC) than untreated controls. These findings do not agree entirely with those reported by Stephenson et. al. (1989) on mature macadamia. They reported consistently lower storage carbohydrates in trunk wood tissues of trees subjected to PBZ during the spring vegetative growth. The fact that this work was conducted on wood samples during a spring flush using only one PBZ rate may explain the variation. In our work leaf samples were collected after the summer flush in trees treated with several PBZ rates.

The trends of sugar levels in relation to PBZ levels in mature trees is rather different from that obtained in macadamia seedlings in earlier work and that reported by Wood (1984) on pecan seedlings. Whilst in the mature trees the lowest PBZ rate resulted in a decrease in leaf sugar levels, in the seedlings the sugar levels were highest at the lowest PBZ rate. This difference is probably due to the fact that in mature trees both vegetative and reproductive growth are competing for assimilates. Cormack and Bate (1976) have shown changes in TNSC levels in macadamia trees for a full season. Their results indicate that higher levels of TNSC are maintained in leaf compared to wood and bark samples throughout the year. They also reported that TNSC levels fall in spring, coinciding with flowering and a growth flush. Stephenson et. al. (1989) associated a decline in carbohydrate reserves in mature trees in spring with the demand by the developing crop rather than the small spring vegetative flush. Whether the developing crop is still a stronger sink than the more vigorous summer flush is an unresolved question.

Data on leaf sugar levels and reproductive and vegetative parameters exhibit interesting patterns. At the lowest PBZ rate, flush growth was stimulated as a result of

development of longer internodes. The kernel recovery and grade 1 kernels were very low in those trees and so were reducing and non-reducing sugars. The low kernel recovery and grade 1 levels reflect poor nut filling and low oil accumulation which are enhanced by adequate carbohydrate reserves in the plant. Since trees treated with low PBZ levels had low sugar levels, these vital plant processes could not be adequately enhanced. Another factor in this context is competition for assimilates between reproductive and vegetative growth in those trees. Due to stimulated flush growth and the strength of the sink in these flushes, most assimilates may have been translocated to the flushes at the expense of nut filling and oil accumulation.

At the higher PBZ rates, flush growth was significantly reduced, while sugar levels were either significantly increased or remained at the same level as in control plants. Likewise the kernel recovery and grade 1 kernels were higher. This is perhaps an indication of remobilisation of sugars from vegetative to reproductive growth as a result of reduced vegetative growth. The high negative correlation between flush length and % grade 1 kernels indicates that reductions in flush length resulted in corresponding increases in grade 1 kernels. Perhaps this is an indication of the remobilisation of carbohydrates between the two sinks.

The increased yield, reductions in flush growth (which should eventually result in reductions in tree size), and improvements in nut quality characteristics are encouraging results of PBZ treatment. Although treatment over more years will be required before the effects of PBZ can be assessed, these early results suggest that it should be possible to control macadamia tree size and achieve a compact stable tree without significant yield losses. The increases in kernel recovery and grade 1 kernels following application of high rates of PBZ represent an added value to the crop and if sustained would result in important increases in farmer returns since in Malawi the macadamia crop is sold at a price based on these two quality factors so that optimal returns are not necessarily achieved simply by maximising yield.

It is evident that shoot growth was reduced only by the application of higher rates of PBZ (3g a.i and above). Lower rates stimulated growth and may have presented a competitive advantage with reproductive growth for assimilates resulting in poor nut quality.

# CHAPTER 7.

# EFFECTS OF PACLOBUTRAZOL ON SEEDLING ROOT GROWTH

## 7.1. INTRODUCTION

Roots serve the important function of water uptake from substrates and the absorption of inorganic ions and control uptake and transport to the rest of the plant. Roots can also synthesize hormones (Sitton, Itai and Kende, 1967) which move to the aerial parts of the plant, influencing the rates and character of shoot development. In addition to these functions, which are common to all root systems, the root may also provide a means of vegetative propagation by formation of adventitious buds. In some species the root may develop nodular structures in response to microorganisms such as Rhizobium and other bacteria, actinomycetes, or blue green algae, associations which lead to symbioses of importance in the fixation of atmospheric nitrogen.

The importance of the root system in tree crops such as macadamia may be underestimated. Management practices to encourage tree growth such as irrigation, fertiliser and herbicide use, and mulching all assume that root systems are adequate. However, from root studies conducted on macadamia by Shigeura and Bullock (1973) it was observed that many factors could affect the adequacy of a root system, which in turn affected the nutrition of the tree. Restriction of root growth development caused by mechanical damage, poor seedling stock selections and poor propagation technique all caused constrictions which limited the flow of nutrients upwards as well as that of assimilates downwards to cause total top and root starvation of the macadamia trees. Despite corrective nutrient measures, response and recovery was not as complete as would be expected from trees with unrestricted root development.

Knowledge of the growth and development of roots and their role in physiological and developmental plant processes lag behind our understanding of aerial plant organs. This can be attributed partly to technical difficulties inherent to root research,

particularly the difficulty in observing root growth non destructively. Probably as a result of this lack of knowledge there is little consensus at present on what constitutes an ideal root system for any particular crop or set of soil conditions.

Success in improving crop productivity through controlling root growth must inevitably be delayed until agronomic information is forthcoming on what changes in root characteristics will benefit yield or efficiency. Conflicting views occur over this issue. Troughton (1982) argues that for young grass plants such as Lolium perenne, increases in shoot growth under a variety of conditions and by different genotypes are associated with less root growth, implying that for heavy shoot yields smaller root systems are required. Passioura (1972) has shown that a smaller root system in wheat is conducive to more even extraction of soil water during the growing season. This helps avoid drought at 'grain-fill' that can severely depress yield. On the other hand, Taylor (1982) considers that for soybean under conditions where yield is limited by water shortage, a deeper (but not necessarily larger) root system could be an advantage.

Constraints to nutrient uptake may be overcome by maximising the absorptive surface area of the root system. This involves attention to root length, radius and fineness of divisions of the absorbing structures which will determine access to nutrient-holding soil pores and particles. A finely divided root system with abundant root hairs is a simple but efficient device for increasing the absorptive area. Weak root systems may develop some devices to compensate for their weakness: 'specialised roots such as mycorrhizas increase uptake of efficiency, and parasitic plants have haustoria which exploit the uptake efficiency of the host.

The absorptive area may be enlarged directly through increased fineness of the root system and proliferation of long root hairs. This reaches its greatest development in

the root clusters of Proteaceae (*Proteoid* roots) (Purnell, 1960), Restionaceae (*Capillaroid* roots), and Cyperaceae (*Dauciform* roots) (Lamont, 1974).

The earliest description of cluster/proteoid roots was by Purnell (1960) who noted dense clusters of rootlets of limited growth which appeared along lateral roots of many members of the family Proteaceae. The rootlets formed on branched or unbranched axes depending on the species. They were crowded together in longitudinal rows which formed opposite the protoxylem poles of the root; had normal primary structure and no secondary growth; formed long root hairs which were frequently distorted or forked at the tip and were relatively ephemeral. This discovery led to several studies aimed at understanding the anatomy, physiology and functions of such root structures. Cluster/proteoid roots have now been reported in several genera of Proteaceae including Banksia, Grevillea, Adenanthos, Conospermum, Hakea, Isopogon, Petrophile, Dryandra, Franklandia, Lambertia, Strangea, Stirlingia, and Synaphea (Lamont, 1974), and other families such as Casuarinaceae (Racette, Louis and Torrey, 1990), Fabaceae (Walker and Pate, 1986), and Cyperaceae (Davies, Briarty and Rieley, 1973).

### 7.1.1. **Factors affecting cluster root development**

Several factors including microorganisms, organic matter, nutrition and aeration have been reported to affect cluster root development. Malajczuk and Bowen (1974) reported that proteoid roots in seedlings of Banksia grandis were caused by non-infecting rhizosphere microorganisms. This was supported by Lamont (1974) who reported proteoid roots on plants grown in soil with non sterile soil suspensions while those under sterile conditions lacked proteoid roots. However, suspensions of Aspergillus, Rhizoctonia spp, Penicillium, and Streptomyces did not induce proteoid root formation. Gardner, Barber and Parberry (1982) working on Lupinus albus reported that microorganisms did not appear to be a prerequisite for proteoid root formation even though it was markedly enhanced by their presence.

Three possible effects of microorganisms in proteoid root production are; firstly, rhizosphere microorganisms may produce substances directly resulting in lateral root initiation and proteoid root development; secondly, they may produce compounds that affect a plant's growth rate and thus its demands for nutrients such as phosphorus; thirdly, the efficiency of cluster roots may be reduced by microbial metabolism of exudates in the rhizosphere and therefore more cluster roots would be required under non-sterile conditions to achieve same results as in sterile conditions. Lamont, Brown and Mitchell (1984) reported that applications of Chloromycetin (an inhibitor specific to prokaryotes) reduced proteoid root production by 37%, giving tentative indications for the involvement of a chemical co-factor of biological origin in stimulating root formation.

Nutrient availability and soil or media type seem to have profound effects on cluster root development. Lamont (1973), working on Hakea prostrata and H. laurina, showed that the level of organic matter, with its associated control over nutrient availability especially in sandy soils was a major determinant of the level of cluster/proteoid root production. More proteoid roots developed in soils with lower organic matter and interaction of low nitrogen and phosphorus resulted in much higher proteoid root production. Further research on Hakea (Lamont 1972a: 1973) indicated that the greatest concentration of proteoid roots occurred in the horizon just beneath the layer of leaf litter, with the concentration decreasing exponentially with depth.

Early reports indicated that proteoid root development may be a response to P deficiency and that these roots were efficient in absorbing P (Purnell 1960). Walker and Pate (1986) noted that increasing P in seedling progenies of Viminaria juncea resulted in 2-3 fold increase in % plant dry weight as nodules and a sharp decline in proportional mass as cluster roots. Dinkelaker, Romheld and Marschner (1989)

reported a response to P deficiency in white lupin (Lupinus albus) by development of proteoid roots which accounted for about 50% of the root dry weight. The proportion of the root system comprising proteoid roots decreased with increases in P and iron supply.

Racette et al (1990) found that aeration, phosphorous supply and nitrogen source markedly influenced the amount of cluster root formation in Gymnostoma papuanum (Casuarinaceae) grown in water culture. Aerated cultures had significantly more cluster roots than non-aerated roots, suggesting that the formation of cluster roots may have a higher requirement for oxygen than that required for normal root growth.

### 7.1.2. **Aim of study.**

Apart from occasional passing reference to cluster roots or proteoids being observed in Macadamia (Firth, 1987), there have been no reported investigations as to their occurrence. This is probably because they seem to occur less extensively than in genera such as Hakea where dense mats of proteoid roots have been reported (Lamont, 1972b). In addition most of the research in macadamia is on commercially managed plants which often grown under heavy management regimes in which the high levels of nutrition might be expected to depress cluster root development.

There also no reports of hormonal involvement in cluster/proteoid root development in all the cases reviewed here. The only involvement being in so much as hormones affect general root development.

This study was aimed at:

(a) establishing the occurrence of cluster roots in macadamia seedlings,

(b) investigating the effects of paclobutrazol on the development of such roots, and

(c) investigating effects of the chemical on carbohydrate levels in the roots.

## 7.2. MATERIALS AND METHODS

Root studies were conducted on 7 month old plants as detailed in Experiment 2 of Chapter 5. In addition, another experiment was set up with paclobutrazol being applied to 7 week old seedlings at rates of 0, 10, 20, 40, and 80 mg per plant on 16$^{th}$ June 1991. The experiment was set up in a single latin square with five treatments replicated five times. Root assessments in this experiment were conducted nine weeks after application of PBZ.

Root length was estimated on the seven month old seedlings (untreated plants and those receiving the highest PBZ rate only) using a method developed by Newman (1966) incorporating modifications by Marsh (1971). This is an indirect method of root length determination commonly referred to as the 'intersection' method. Briefly, roots were cut into short straight sections and submerged in 5 ml of water in a vessel with a clear base. The vessel was placed on a grid with 1cm sq. lines. The intersections between the root and the grid lines were then counted and added up. Root length was determined using the following formula:

$$\text{Root length (cm)} = N \times {}^{11}/_{14} \times \text{Grid units}$$

Where N = number of intersections.

For 1 cm. sq. grid the ${}^{11}/_{14}$ X Grid units was 0.7857 as calculated by Tennant (1975).

Root clusters were carefully excised and washed, then the number of clusters was counted without scoring for size or length. The lateral roots which comprised an individual cluster were too numerous and fine to permit individual measurement. The roots were then oven-dried at 105$^0$C for 24 hours to determine dry weight. For carbohydrate determination, roots were oven-dried at 95$^0$C for one hour followed by four hours at 70$^0$C prior to analysis as detailed out in Chapter 2.

## 7.3. RESULTS

Dry matter:

There were significant differences (P = 0.01) between paclobutrazol treatments in root dry weight of the 7 month old seedlings. However there were no significant differences in root dry matter accumulation in the 4 month old seedlings (Table 7.1 and Appendix 2G (a)). No clear patterns could be established as to the effect of PBZ on accumulation of dry matter in the root. This was compounded by high variation in root dry weights between plants. There were no significant differences in overall root length between control plants and those at the highest PBZ rate (Table 7.1).

Cluster roots;

Cluster roots were generally observed only on the macadamia seedlings treated with paclobutrazol. A typical cluster on the root axis appeared short (25-30mm) with longitudinal rows of determinate rootlets along the lateral root (Plates 7.1 to 7.5). The clusters often arose on or off a long non-cluster root, in association with the current season's growth of the parent root in a lateral relationship. In some cases, long non-cluster roots developed within the cluster root axis forming an adventitious relationship. Young clusters had dense fine root hairs, on their rootlets, which collapsed when exposed to the atmosphere. Root hairs were not present on rootlets on older clusters. The old clusters were brown and clung firmly to litter and soil particles so that a mature cluster often looked like a piece of soil attached to the root.

PBZ application significantly influenced cluster root development in both 4 (P=0.05) and 7 (P=0.001) month old plants (Table 7.1, Fig. 7.1 and Appendix 2G (b)). In both cases untreated controls had no cluster roots at all while all treated seedlings had many clusters. While untreated controls had no cluster roots, increased levels of PBZ resulted in increased cluster root development and once again the seedlings treated at the highest PBZ rate had the highest numbers of root clusters.

Table 7.1 Effects of PBZ application on root dry matter, root length and number of cluster roots in 4 and 7 months old seedlings. PBZ was applied at 2 and 5 months of age respectively.

| Parameter | plant age (months) | mg. Paclobutrazol per plant | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 20 | 40 | 80 | SED | 5%LSD | |
| Dry weight (g) | 4 | 1.83 - | | 0.99 | 1.30 | 1.67 | 1.84 | 0.44 | | |
| | 7 | 1.81[a] | 3.07[b] | 2.97[b] | 2.04[a] | 3.46[b] | 1.62[a] | 0.36 | 0.72 | ** |
| No. of clusters* | 4 | 0[a] | - | 4.2[b] | 4.4[b] | 17.8[c] | 16.3c | 1.21 | 2.57 | *** |
| | 7 | 0[a] | 1.0[a] | 2.9[b] | 3.2[b] | 4.8[c] | 6.9[d] | 0.56 | 1.11 | *** |
| Root length (cm) | 7 | 515 | - | - | - | - | 527 | | NS | |

Means in the same row with the same superscript are not significantly different at P=0.05.

* Values are weighted means derived by square root transformation of original data, followed by squaring of the means.

**Fig. 7.1 Trends in cluster roots formed in 4 and 7 month old plants following treatment with PBZ at 2 and 4 months.**

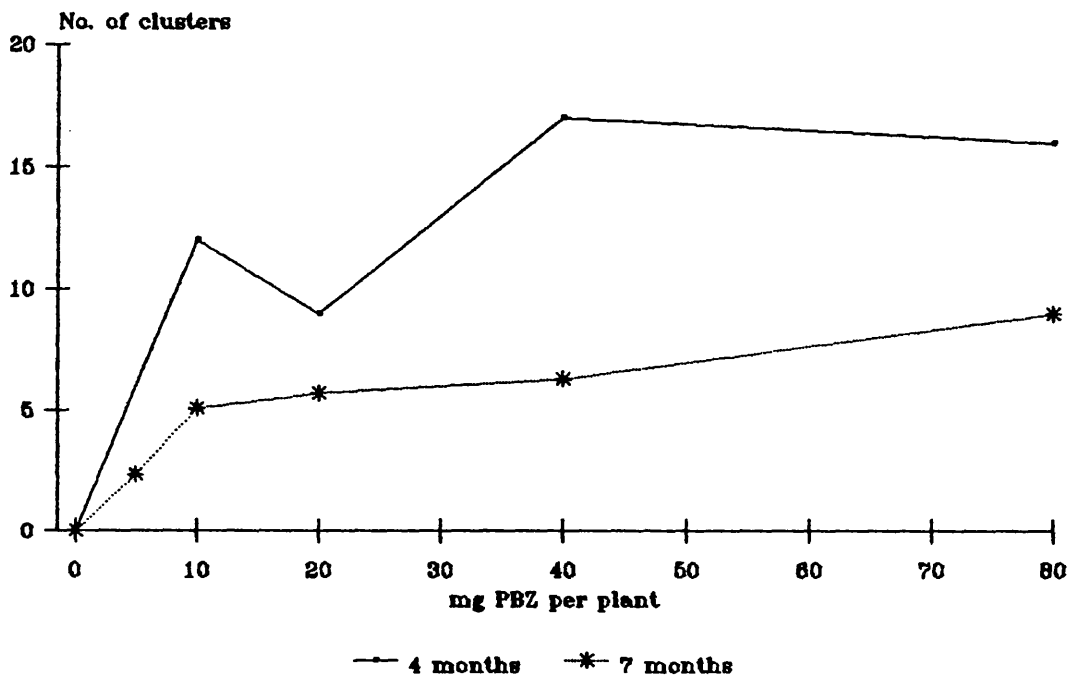



Plate 7.1. A section of a typical non-cluster root, showing the sparse secondary roots and the thick growing end (**a**)

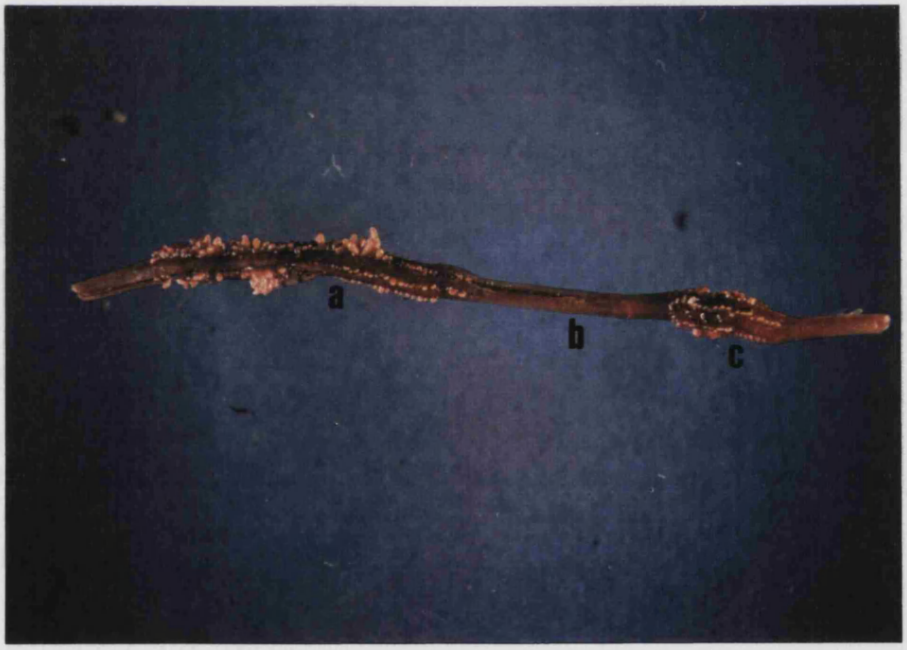



Plate 7.2. A section of macadamia root axis in the early stages of cluster development. Note the single longitudinal rows of rootlets in the two cluster root sections (**a** and **c**) interspaced with a non-cluster root axis (**b**)

(a)

(b)

Plate 7.3. Cluster root axes showing **(a)** short dense rootlets and **(b)** long rootlets.

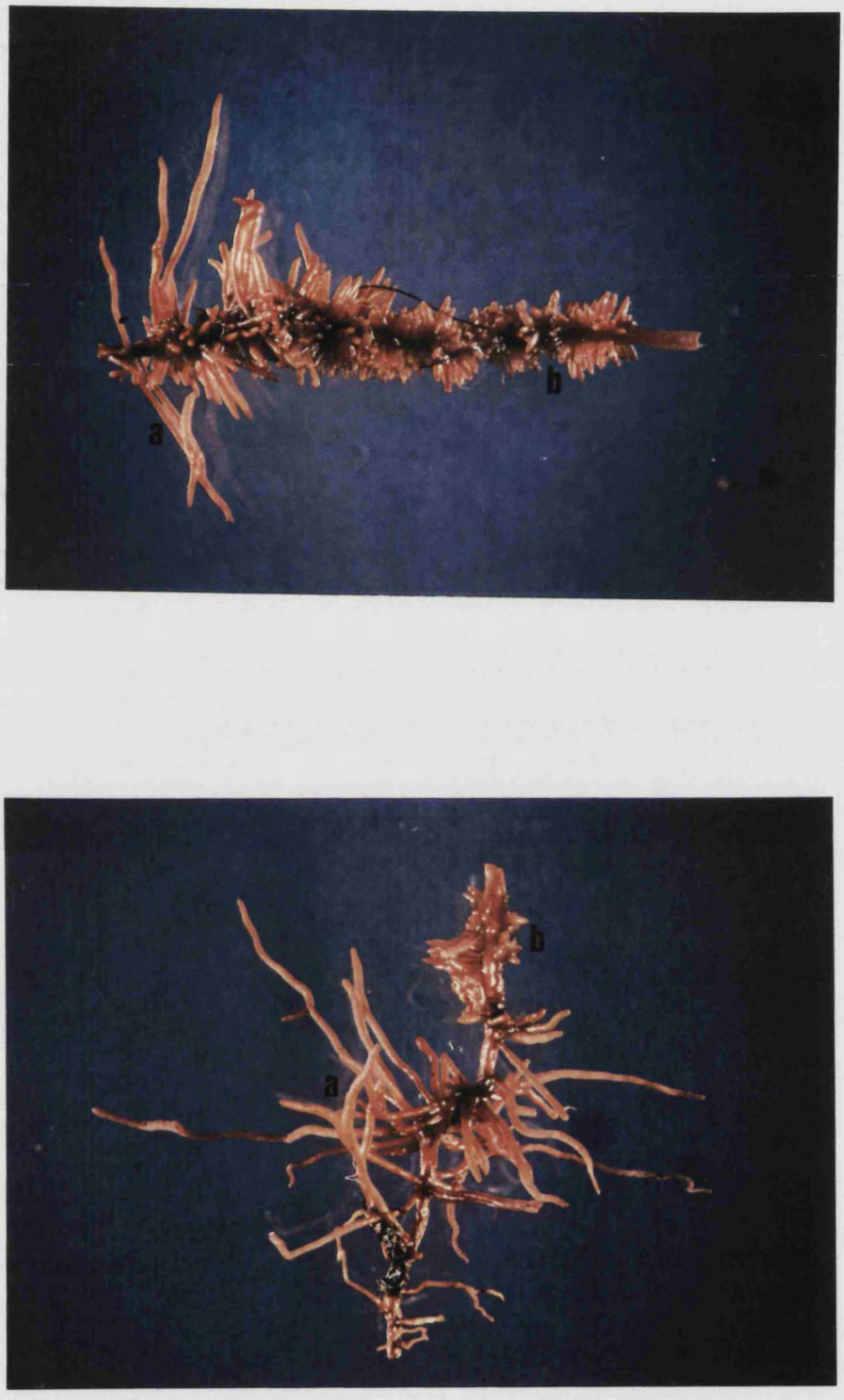



Plate 7.4. Cluster root axes showing linear profiles of both **(a)** long and **(b)** short rootlets on the same axis.

(a)

(b)

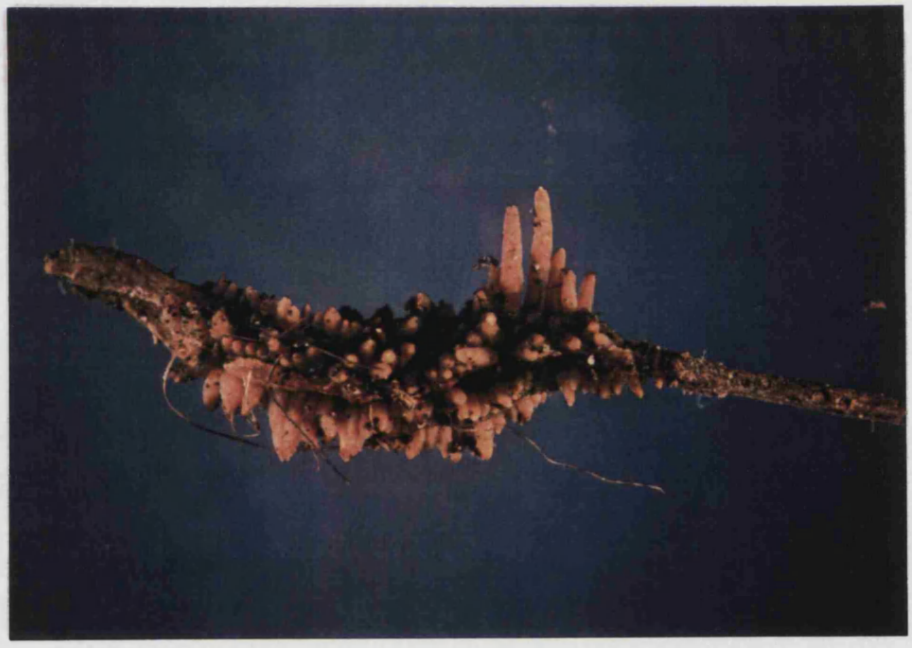


Plate 7.5. Old mature cluster roots showing **(a)** browning and **(b)** tendency to cling to litter particles.

The data clearly show that the 4 months old plants had the most cluster roots. It should be noted that these plants were treated with PBZ when they were 2 months old while the 8 and 10 month old seedlings were treated at 4 months. Plate 7.6 shows roots and clusters from four months old plants from one replicate treated with various levels of PBZ.

Carbohydrates:

PBZ application had significant effects on root reducing sugar ($P = 0.001$) and starch ($P = 0.05$) concentration (Table 7.2, Fig. 7.2a-c and Appendix 2G (c)) of 4 months old plants. Roots from plants treated with the lowest PBZ rate (10mg) had the lowest reducing sugar levels, while higher PBZ rates resulted in sugar levels higher than those of the control. All treated plants had lower root starch than untreated controls. Plants treated with the lowest PBZ rate resulted in the lowest starch level. PBZ application had no significant effect on root non-reducing sugar levels although there was a trend of an increase in sugar levels with increased PBZ rates.

Plate 7.6. Normal and cluster roots (excised) obtained from 7 month old seedlings following treatment with Paclobutrazol at 5 months of age. PBZ treatments were as follows; (a) 0, (b) 5, (c) 10, (d) 20, (e) 40, and (f) 80 mg PBZ plant. Note the abscence of cluster roots in plants treated with 0, 5, and 10 mg PBZ.


a
b
c
d

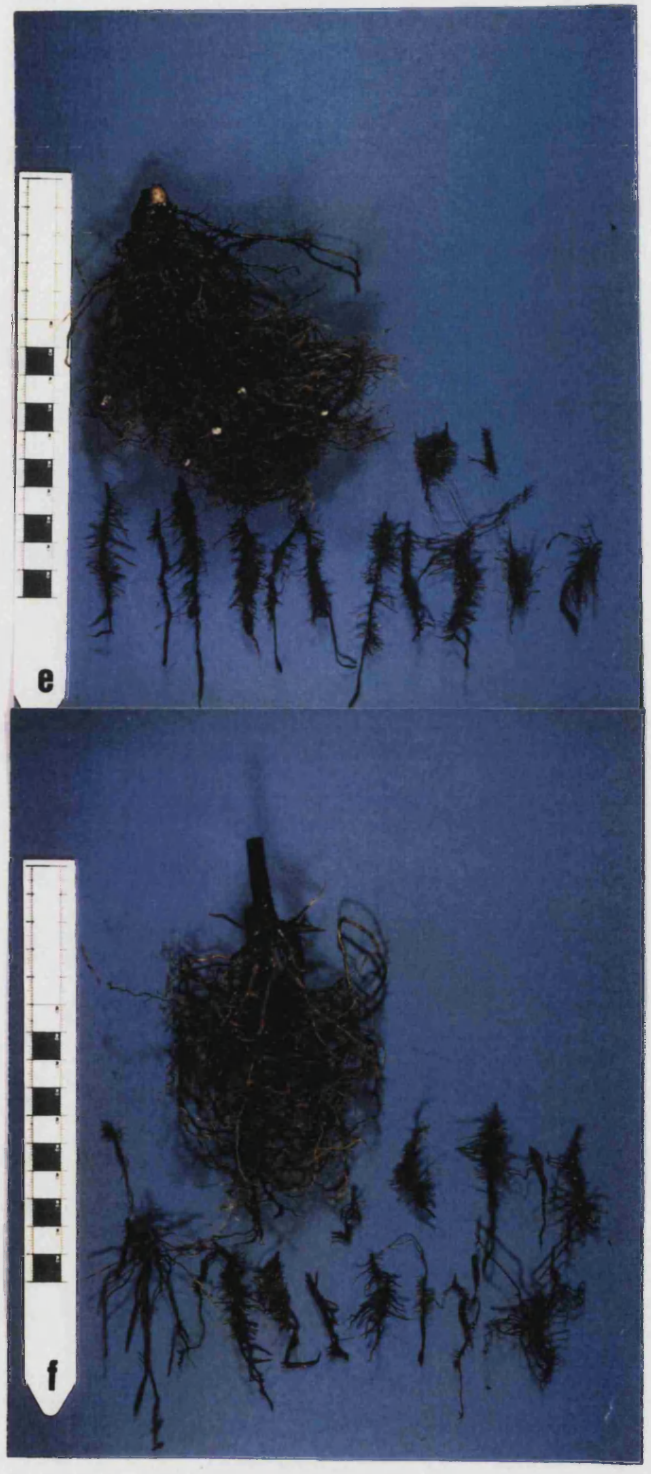

e
f

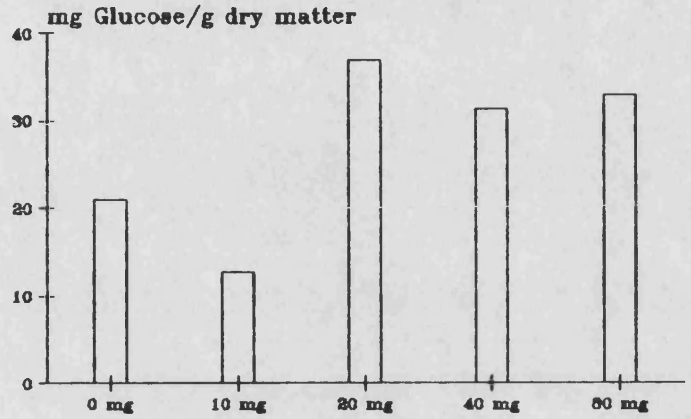
Table 7.2. Root carbohydrate levels on four months old macadamia seedlings following treatment with PBZ at two months of age.

| | Glucose equivalents (mg/g dry weight) | | |
|---|---|---|---|
| PBZ | Reducing | Non-reducing | Starch |
| 0 | 20.89$^{b}$ | 10.43 | 83.27$^{c}$ |
| 10 | 12.72$^{a}$ | 10.47 | 62.93$^{a}$ |
| 20 | 36.93$^{e}$ | 17.56 | 71.64$^{ab}$ |
| 40 | 31.29$^{c}$ | 21.23 | 73.43$^{b}$ |
| 80 | 32.86$^{d}$ | 23.39 | 71.58$^{ab}$ |
| | *** | NS | * |
| SED | 0.46 | 5.18 | 3.18 |
| 5% LSD | 1.27 | | 8.83 |

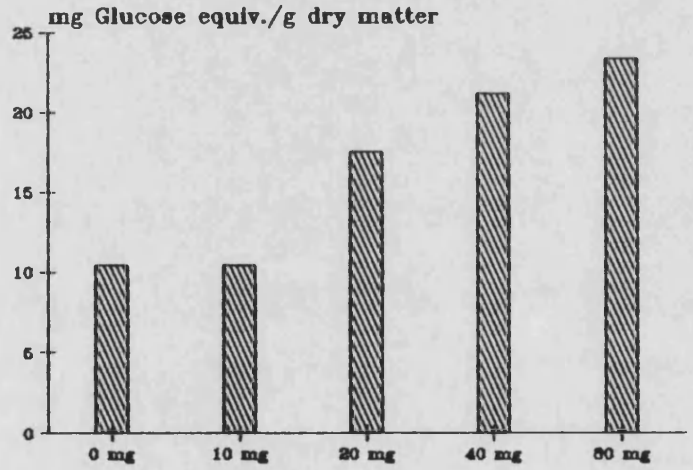
Means in the same column followed by same superscript are not significantly different at P = 0.05%

* P = 0.05 ** P = 0.001

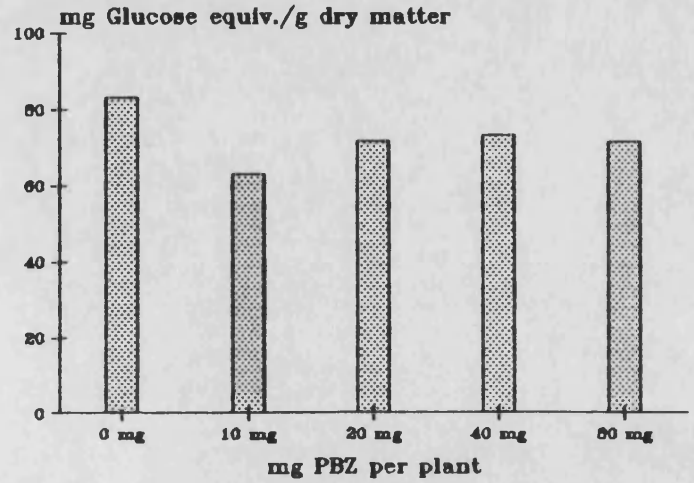
Fig. 7.2 Levels of (a) reducing, (b) non reducing sugars and (c) starch in roots of 4 month old plants treated with PBZ




(a) Reducing sugars




(b) Non-reducing sugars




(c) Starch

## 7.4. Discussion

It is clear that the application of PBZ had no influence on dry matter accumulation in the root as expressed by root dry weight. However, although root weight is commonly used for evaluating effects on root growth it is not a particularly effective parameter for characterising the amount of functional root system. The assumption that root weight is correlated to some kind of root activity may not be valid, since fine roots represent a small fraction of the total weight although they may be the most active part of the root system. Hence root weight really should not be considered a very good parameter for estimating root activity especially in macadamia where root systems are generally small and variable.

It is interesting to note that PBZ application had significant effects on the number of root clusters and that all untreated plants had no root clusters at all. Cluster or proteoid roots are almost universally present in the family Proteaceae (Lamont 1982). However, no investigations have been reported on the formation of these roots in macadamia although they have been observed (Firth, 1987). The structure of the clusters observed here seem to be similar to that reported in other species of the Proteaceae such as Hakea (Lamont, 1972b) and Grevillea (Purnell, 1960). However they do not appear to be as dense and abundant as reported in the other species. Unlike the situation in Hakea, the clusters in macadamia are not of uniform length.

The effect of PBZ on the number of root clusters suggests that endogenous gibberellin levels might be directly or indirectly involved in the initiation. The role of gibberellins on root development seems to be unclear. Most reports indicate that gibberellins inhibit root growth (Stowe and Yamaki, 1959) or have no effect at all (Blakely, Rodaway, Hollen and Croker, 1972). The apparent inconsistencies of these observations probably resulted from the deduction that effects observed in detached

roots in vitro may not be representative of root responses in situ. Reports indicate that initiation of adventitious roots is generally inhibited by gibberellins (Nickell, 1982). However, very low concentrations ($10^{-11}$ - $10^{-7}$M) of $GA_3$ promoted root initiation in pea cuttings and pine when supplied at the first observable stage of root initiation. $GA_3$ may act indirectly to improve rooting by causing reversion to juvenile phase growth on which adventitious roots are more readily formed. Haissig (1972) reported that applied GA reduced the number of primordia and also the number of cells per primordium in brittle willow (Salix fragilis L) stems.

Since PBZ is known to inhibit the metabolic pathway by which gibberellins are synthesized in plants (Rademacher et. al., 1984), it is likely that high levels will tend to reduce endogenous GA levels and this may be responsible for the increase in adventitious root initiation. The higher cluster numbers on 4 months old compared to 7 month old seedlings probably resulted from application of PBZ at a stage when roots had plenty of growth primordia or had lower GA levels. It should be noted that PBZ was applied at 2 and 4 months of age for the 4 and 7 months old seedlings respectively. Lamont (1972b) has reported that in Hakea, old proteoid roots are brown, desiccated and shed their root hairs. It is also likely that after some time cluster roots completely collapse and this may explain the higher number of cluster roots in younger plants compared to older ones.

The relationship between roots and plant growth regulators is rather complicated. Phillips and Jones (1964) and Sitton et. al., (1967) reported that cytokinins and some gibberellins can be synthesized in the roots. Further evidence shows that seedling roots probably contain auxin, cytokinin, gibberellin, absiscic acid and low levels of ethylene (Torrey 1976). Most research has shown that roots are usually sensitive to applied hormones. Blakely et. al. (1972) reported massive responses to auxin by formation of numerous branch roots in Haplopappus ravenii root segments. The initiation of the branch meristems was reversed by cytokinin. Gibberellic acid had no

effect on root initiation. Brian, Hemming and Love, (1960) concluded that gibberellic acid inhibited formation of root meristems in mature stem tissues of cuttings. Butcher and Street (1960), on the other hand found that gibberellic acid increased the number of emergent laterals in tomato root cultures. All indications are that PBZ induced cluster root development by offsetting the inhibition of the formation of root meristems by endogenous GA. Presumably the lower PBZ rates were not adequate to offset the hormonal balance. Whatever the case this result suggests that hormones are implicated in cluster root development in macadamia seedlings.

The development of cluster roots is an important phenomenon in macadamia as they may help with water and nutrient absorption and also in the modification of the rhizosphere. The absorptive root surface area is enlarged directly through increased fineness of the root system and proliferation of root hairs. Apart from cluster/proteoid roots, the *capillaroid* roots of Restionaceae and *dauciform* roots of Cyperaceae have similar well developed forms. Dell, Kuo and Thomson (1980) studied proteoid roots on seedlings of Hakea obliqua in water culture and reported that, on average, 960 rootlets were produced on each proteoid representing a 25 fold increase in surface area over parent root. Lamont, Brown and Mitchell (1984) reported that proteoid roots of Leucadendron laureolum accounted for 40% of the root mass of 9 month old plants. The surface area per gram dry mass is about 15.8 times higher for proteoid roots than non-proteoid roots, the reduction in width of rootlets and greater length and density of root hairs (Lamont 1982) being largely responsible for this increase in absorptive area. The massive increase in root area indicates the potential of this type of root structure for localised enhancement of nutrient uptake. In addition since root clustering favours the maintenance of a moist rhizosphere, the rate of nutrient release from soil particles will be both maximised and prolonged compared with unclustered roots. Indeed some research has shown enhanced nutrient absorption by proteoid roots over non-proteoid roots. Jeffrey (1967) reported a significantly greater uptake of

labelled phosphorus ($^{32}P$) by proteoid roots compared to non-proteoid roots in Banksia.

There are also reports of considerable modifications of the rhizosphere around proteoid roots in terms of mineral balances and soil structure. Research on Lupinus albus (Gardner, Parberry and Barber, 1980; 1982: Gardner, Barber and Parbery, 1982) demonstrated considerable modification of the soil in the vicinity of proteoid roots with significant effects on pH, Mn and Fe reduction. Similar effects were also reported by Dinkelaker et. al. (1989) on L. albus where the pH in the rhizosphere soil of the proteoid root zones dropped to 4.8 and abundant white precipitates, shown to be citric acid, became visible after 2 weeks. Citric acid is highly effective in dissolving sparingly soluble phosphates such as tricalcium P or Fe and Al-Phosphates. Hence it might be that plants with proteoids thrive in low P soils because they utilise sparingly soluble P sources.

The effects of PBZ on carbohydrate levels in the roots may have a lot to do with shoot growth and the resultant demand. The low reducing sugar and starch levels in the root system of plants treated with the lowest PBZ rate was probably due to shoot growth which was not very restricted in these plants. Hence there may be a competitive interaction between root carbohydrate levels and the increased demand in the developing shoot. In plants treated with high rates of PBZ, large amounts of reducing sugar seem to be present in the roots, although surprisingly the level of storage sugars is lower.

Similar relationships between root and shoot growth and assimilate distribution have been reported elsewhere. Williamson and Coston (1989) reported that in peach trees a decrease in production of new roots coincided with major flushes of shoot growth. They concluded that reduction in root growth was likely to be due to the internal factors that influenced distribution of assimilates between shoots and roots. Shoots

seem to provide a more competitive sink for photosynthates than roots during periods of rapid shoot growth. However, the amount of shoot growth that can occur without subsequent root growth is limited by the ability of root system to supply the shoot with water, nutrients and hormones. This creates or contributes to the creation of a cycle of growth phases between shoots and roots.

The development of cluster roots in macadamia is a phenomenon which should be encouraged. This is particularly important as macadamias develop a rather poor root system which might restrict shoot growth. The implication of some form of hormonal control on cluster root development which has been reported here has not been reported elsewhere.

CHAPTER 8

# GENERAL DISCUSSION

Each chapter has already been discussed separately. In this section, an attempt will be made at bringing together all relevant points, highlight the achievements, and also indicate sections which need further investigation. The control of vegetative growth in macadamia is of great importance in Malawi where prevailing environmental conditions favour excessive vegetative growth. Benefits from control of such growth are enormous and include the development of compact trees which are easy and cheaper to manage requiring less inputs such as labour, fertiliser, water and pesticides; increases in plant populations from reduced spacings which may eventually lead to increased yield per unit area; and the possibility of assimilates being partitioned to reproductive growth (following control of vegetative growth at the right time) resulting in improvements in yield and quality.

The research reported here has confirmed the occurrence of more or less continuous flushing in macadamia seedlings first reported by Cormack and Bate (1976). Unlike mature trees, where major flushing peaks occur once or twice a year, seedlings flush more frequently with short intervening dormancy periods of 1 to 2 weeks. The results show the strong possibility of such flushes being caused by some endogenous factors as proposed in Oreopanax (Araliaceae) by Borchert (1969) and in cocoa (Theobroma cacao) by Greathouse et. al. (1971). Macadamia seedlings maintained flushes even under the controlled growing conditions of environmental growth cabinets.

However, the shoot and root growth relationships, in particular the relatively poor root development and vigorous shoot development in seedlings, could influence the endogenous rhythms and hence affect flushing. This could be as a result of low levels of cytokinins produced in the roots in relation to ABA levels in the above ground

parts. The inhibitor - promoter balance has been reported to influence flushing in cocoa (Alvim, et. al., 1974). With the type of growth observed in macadamia seedlings, it should be relatively simple to reach the critical leaf area reported in cocoa where the ABA action totally overcomes cytokinin effects and leads the plant into dormancy. Since macadamia seedlings do not shed their leaves, this stage of correlative inhibition could be overcome by induction of abscission layers to reduce movement of leaf ABA to the shoot tip or through the growth of more roots which may lead to production of more cytokinins and offset the balance and trigger a shoot flush. It is, therefore, possible that there are two types of growth flushes occurring in these plants, one involving the shoot and the other involving the roots which complement each other.

The shoot flushes could also be influenced by changes in plant water balances. Since the shoot grows more than the root, it is possible that the root system can not then sustain the demand for water posed by the shoot. This disturbed water balance may be responsible for the arrest of growth (Greathouse et. al., 1971) until the absorbing surface is increased resulting in more vegetative growth. It is also possible that the changes in internal moisture balances could also affect ABA and cytokinin levels in the shoots (Wareing, 1970) and influence shoot flushes. The defoliation experiment suggests that the pattern of shoot growth can be temporarily disturbed particularly by the removal of young leaves, This probably suggests the existence of feedback mechanisms limiting shoot leaf growth which may be linked to root development according to a model developed by Borchert (1973).

It is possible that flushing has an endogenous component, which is subject to modulations by response to environmental effects. A full understanding of the physiological control of flushing must await determinations of the changes in endogenous inhibitors and promoters within shoot systems during flushing cycles. Further work needs to be conducted on the pattern of root growth in relation to

shoot flushes. Possibilities include studies in transparent containers to map root growth, or the growing of plants using nutrient film technique where root growth can be easily followed.

The results also show the partitioning of assimilates with flush growth. Sugar and starch levels were highest in flushing plants and lowest in plants at bud break. The very high levels of TNSC's in flushing plants were as a result of high levels of both reducing and starch levels in flush leaves. It is apparent that the flush leaves at this time were a strong sink. This would confirm previous findings by Allan (1972) that expanding leaves in macadamia seedlings were a very strong sink. This is further supported by the very low TNSC levels in mature leaves just below the flush leaves, which in this case may have acted as a source for the strong sinks above.

Macadamia seedlings generally seem to have low levels of non-reducing sugars in their leaves, but high levels of starch and reducing sugars. As the seedlings grow, there are indications of starch accumulation in the leaves. This is probably because the older plants have more leaves, and hence are more active photosynthetically and also because older plants have a lower flush frequency and are, therefore, capable of building up carbohydrate reserves. Generally roots seem to have low starch and reducing sugar levels, perhaps indicating that root reserves may not be necessary to sustain flush growth although this does not preclude the effect of root hormones or moisture absorption capacity in influencing shoot flushing. Carbohydrate determinations were not conducted elsewhere in the plant other than the roots and leaves. Stephenson et. al. (1989) have reported accumulation of high starch levels in the bark of mature trees. If this is also the case in seedlings, then perhaps the high TNSC levels in leaves and roots of flushing plants are as a result of mobilisation of the bark reserves. The low levels in dormant plants are probably because most of the starch was stored in the bark and not leaves. Further research could assess carbohydrate levels in all plant parts including the bark and even wood.

Applications of paclobutrazol to both seedlings and bearing trees had profound effects on shoot extension growth. In both cases the effect seemed to be through the effect of PBZ on internode elongation. In seedlings, application of high PBZ rates resulted in reduced shoot extension growth. However, applications of the lowest rate of PBZ resulted in stimulation of shoot growth. This has also been reported in peach seedlings by Liyembani and Taylor (1989). The causes of growth stimulation are not very clear but could be due to sensitivity to PBZ at the time of application. Seedlings treated at 3 and 5 months of age gave conflicting results in this aspect, hence the phase of plant growth may be important specially where low rates of PBZ are used. The concept of the control of growth and development of tissues being subject to sensitivity of the tissue to the hormone has been advocated by Trewavas (1981).

PBZ application had no effect on leaf sizes but influenced the number of leaves produced as a result of the short internodes. This could mean increased shading among leaves and possible reductions in photosynthetic abilities. In bearing trees, PBZ reduced shoot growth through reduced internode elongation in both seasons of application. In the first year following application, there was stimulation in shoot growth in trees receiving the lowest rate of PBZ. This is similar to the trend in seedlings and perhaps the same explanations prevail. The reduction of shoot extension in bearing trees treated with high levels of PBZ is an important finding. It shows possibilities of developing a compact tree which could be easily managed.

It is perhaps a little too much to expect significant yield and quality improvements following treatment with PBZ for only two seasons, especially in trees which have not yet reached their optimum production age. However, the current trends suggest the possibility that yields and quality might improve with time. In any case with reduced shoot growth it is then possible to increase the plant population and obtain increases in yield on a per unit area basis.

Application of high levels of PBZ had no effect on leaf carbohydrates in seedlings. However, at the lowest PBZ rate leaves had the highest carbohydrate levels. This is in agreement with earlier findings that flushing plants had the highest carbohydrate levels. In this case, shoot growth was stimulated in seedlings receiving the lowest rate of PBZ and the creation of a strong sink probably meant mobilisation of reserves to the flush leaves. However, bearing trees receiving the lowest PBZ levels (which also had stimulated growth) had the lowest reducing and non-reducing sugars. This discrepancy between seedling and mature tree carbohydrate levels could be explained in two ways. In mature trees during this time nuts were being harvested, so it is very likely that the developing nuts were also using the assimilates produced in the leaves, leading to low reserves in the leaves. The other possibility is that although shoot growth was stimulated, trees were sampled at the end of the summer flush when the leaves were not as a strong a sink as they were hardening.

It is very likely that during a normal flush growth season there are changes in assimilate partitioning from given leaves or leaf type. This has been shown in vines by Quinlan and Weaver (1970). Knowledge of any such trends in macadamia would be very important as the summer vegetative flush coincides with nut development and may help in determining the timing of PBZ application. Further work on the use of PBZ on both seedling and mature macadamia plants is needed to determine the effect of PBZ on the frequency of shoot flushes. In addition it would be useful to measure the effect of PBZ applied to mature trees, as close to the summer flush as possible.

One of the most interesting results of the research project was the effect of PBZ on the production of cluster roots. While cluster root development has been reported in other species of the Proteaceae (Purnell, 1960; Lamont, 1972a and b: Lamont, 1973), there have been no detailed reports of their occurrence in macadamia, and there has been no report relating cluster root development to plant hormones or plant growth

regulators. Our research has found that application of PBZ resulted in cluster root development in seedlings at all ages used. While the exact functions of cluster roots are not well known, there are reports of their importance in the modification of the rhizosphere and mineral absorption (Jeffrey, 1967; Gardner, et. al., 1980; Dinkelaker et. al. 1989). Certainly the absorptive root surface area seems to be enlarged with the development of numerous fine roots and proliferation of root hairs. This is particularly important in macadamias as they do not develop an extensive root system. PBZ application also increased reducing and non-reducing sugars in the root. This is probably a result of competitive interactions between root and shoot development, as plants treated with low PBZ had high leaf carbohydrates but low root carbohydrate.

Several possibilities exist as to the induction of cluster roots following PBZ application. Firstly, PBZ may affect roots directly by reducing levels of endogenous gibberellins thereby affecting root development by releasing adventitious root primordia. Nickell (1982) has reported the inhibition of adventitious roots by gibberellins. Secondly, PBZ may affect growth of the whole plant, affecting root development indirectly, perhaps not through root gibberellins. In this case perhaps cluster root formation is limited by the availability of root carbohydrate. Since PBZ increases root carbohydrates, the cluster roots are allowed to form. Thirdly, PBZ may have effects on soil microorganisms and this may affect some aspects of the microorganisms - root interaction. Research is required to elicit the mechanisms enabling cluster root development following PBZ application and also whether cluster roots can be induced by PBZ in bearing trees.

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APPENDIX 1 Somogyi-Nelson Colorimetric Method for Sugar Determination.

I. Reagents

(i). Low alkalinity Copper Reagent;

*Constituents;*

(1) 12g Rochelle salt (Sodium potassium tartrate).

(2) 24g anhydrous sodium carbonate ($Na_2CO_3$)

(3) 40ml 10% copper sulphate solution ($CuSO_4.5H_2O$)

(4) 16g sodium hydrogen carbonate ($NaHCO_3$)

(5) 180g anhydrous sodium sulphate ($Na_2SO_4$) in 500ml water

*Preparation;*

(1) and (2) were dissolved in 250ml water, (3) was added while stirring followed by (4). (5) was boiled, to dissolve air, cooled and mixed with the other solution and diluted to 1 litre. the solution was allowed one week standing and the clear supernatant was used.

(ii). Arsenomolybdate colour reagent;

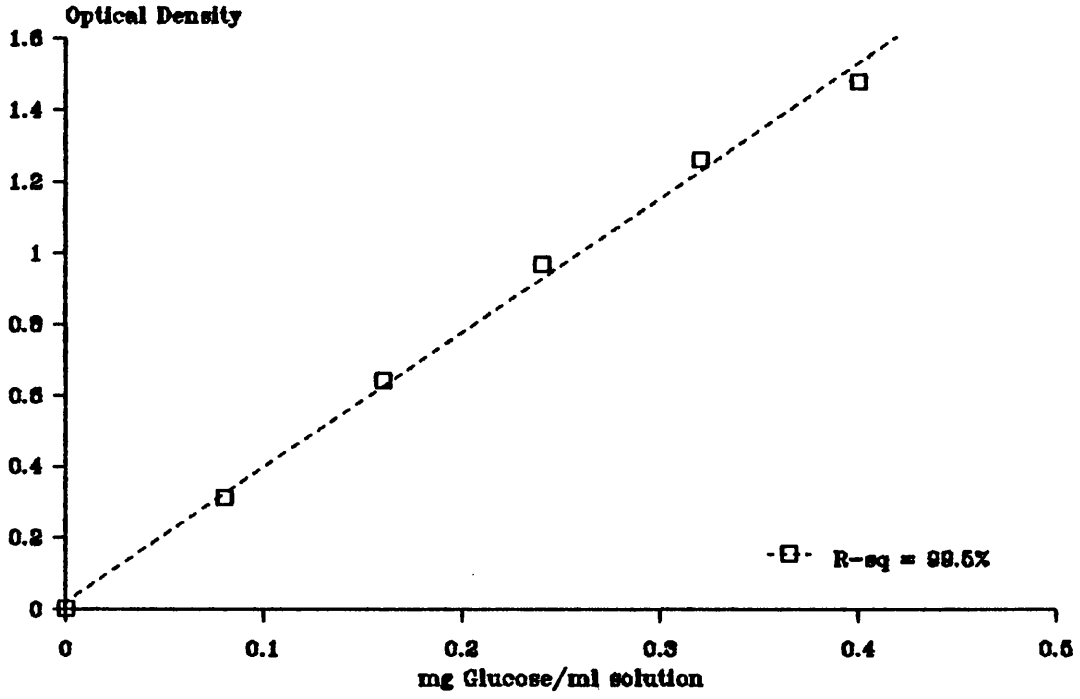
*Preparation;*

25g ammonium molybdate was dissolved in 450ml distiled water, 21ml concentrated sulphuric acid ($H_2SO_4$) added and mixed. 3g disodium hydrogen arsenate heptahydrate ($Na_2HASO_4.7H_20$) dissolved in 25ml water was added to this solution and incubated at $37^0C$ for 24 hours and stored in glass stoppered brown bottles.

II Glucose Standard Solutions:

These were used to provide a standard curve to be used to determine sugar levels (Appendix Fig. 1) from optical densities of test solutions. Working standard solutions of 0.08 and 0.1 to 0.96 and 1mg glucose/ml respectively were made from stock solutions of 0.8 and 1mg/ml anhydrous D glucose.

**Appendix 1. Glucose standard curve used to derive glucose or glucose equivalents from optical densities of test samples.**



APPENDIX 2:

ANALYSES OF VARIANCE FOR DATA IN THE VARIOUS TABLES AND FIGURES

Key:

Significance levels

* P = 0.05

** P = 0.01

*** P = 0.001

SED (standard error of the difference between means) was derived from $\underline{2MSE}$, where MSE is the error mean square, and n is the number of observations in each variable.

LSD (least significant difference) = t(df error) X SED. All LSDs were calculated at P = 0.05.

df = degrees of freedom

SS = sums of square

MS = mean square

Sign. = significance level

NS = not significant

**(2A)** ANOVA for data on decapitation and defoliation of seedlings (Table 3.2).

(a) Stem length

(i) 3 weeks

| Source | df | SS | MS | F value | Sign. |
|---|---|---|---|---|---|
| Treatments | 3 | 2684 | 895 | 8.64 | ** |
| Error | 10 | 1037 | 104 | | |
| Total | 13 | 3720 | | | |

SED = 7.21

LSD = 16.08

(ii) 6 weeks

| | | | | | |
|---|---|---|---|---|---|
| Treatments | 3 | 2291 | 764 | 4.14 | * |
| Error | 10 | 1844 | 184 | | |
| Total | 13 | 4135 | | | |

SED = 9.59

LSD = 21.39

(iii) 12 weeks

| | | | | | |
|---|---|---|---|---|---|
| Treatments | 3 | 1077 | 359 | 1.98 | NS |
| Error | 10 | 1810 | 181 | | |
| Total | 13 | 2887 | | | |

SED = 9.51

(b) Leaf area

(1) 3 weeks

| Source | df | SS | MS | F | Sign. |
|---|---|---|---|---|---|
| Treatments | 3 | 5581 | 1860 | 1.47 | NS |
| Error | 10 | 12657 | 1266 | | |
| Total | 13 | 18238 | | | |

SED = 25.16

(ii) 6 weeks

| | | | | | |
|---|---|---|---|---|---|
| Treatments | 3 | 9919 | 3306 | 1.91 | NS |
| Error | 10 | 17340 | 1734 | | |
| Total | 13 | 27259 | | | |

SED = 29.44

(iii) 12 weeks

| | | | | | |
|---|---|---|---|---|---|
| Treatments | 3 | 17516 | 5839 | 1.26 | NS |
| Error | 10 | 46501 | 4650 | | |
| Total | 13 | 64018 | | | |

SED = 48.22

**(2B)** ANOVA for data on carbohydrate levels of 3 months old plants (Table 4.1)

(i) Reducing sugars

| Source | df | SS | MS | F | Sign. |
|---|---|---|---|---|---|
| Growth phase | 2 | 235.4 | 117.7 | 3.44 | NS |
| Plant part | 2 | 2393.6 | 1196.8 | 34.99 | *** |
| Error | 4 | 137.0 | 34.2 | | |
| Total | 8 | 2766.0 | | | |

SED (Growth phase) = 16.77

SED (Plant part) = 6.43

LSD (Plant part) = 15.76

(ii) Non-reducing sugars

| | | | | | |
|---|---|---|---|---|---|
| Growth phase | 2 | 8.34 | 4.17 | 0.50 | NS |
| Plant part | 2 | 19.64 | 9.82 | 1.17 | NS |
| Error | 4 | 33.67 | 8.42 | | |
| Total | 8 | 61.65 | | | |

SED (Growth phase) = 2.16

SED (Plant part) = 2.43

(iii) Starch

| | | | | | |
|---|---|---|---|---|---|
| Growth phase | 2 | 135 | 68 | 0.29 | NS |
| Plant part | 2 | 865 | 432 | 1.87 | NS |
| Error | 4 | 925 | 231 | | |
| Total | 8 | 1925 | | | |

SED (Growth phase) = 10.86

SED (Plant part) = 14.09

(2C) (a) Fortnightly stem extension growth (mm) on 6 months old plants following PBZ application at 3 months.

| mg PBZ per | Time after PBZ application (weeks) | | | |
|---|---|---|---|---|
| plant | 6 | 8 | 10 | 12 |
| 0 | 39.2 | $56.7^{cd}$ | $83.3^{bc}$ | $95.3^{cd}$ |
| 5 | 50.0 | $75.0^{d}$ | $94.2^{c}$ | $105.8^{d}$ |
| 10 | 44.2 | $52.5^{cd}$ | $72.5^{bc}$ | $76.7^{cd}$ |
| 20 | 33.3 | $50.0^{abc}$ | $58.3^{ab}$ | $65.8^{bc}$ |
| 40 | 25.0 | $30.8^{ab}$ | $35.8^{a}$ | $38.3^{ab}$ |
| 80 | 24.2 | $28.3^{a}$ | $30.0^{a}$ | $31.7^{a}$ |
| SED | 10.3 | 11.7 | 13.7 | 16.02 |
| SIGN. | NS | ** | *** | *** |
| LSD | | 24.1 | 28.3 | 33.0 |

(b) ANOVA on data on effects of PBZ on the growth of 6 month old seedlings

(i) Plant height 3 months (12 weeks) after treatment (Figure 5.2)

| Source | df | SS | MS | F | Sign. |
|---|---|---|---|---|---|
| PBZ | 5 | 26719 | 5344 | 6.94 | *** |
| Replicates | 5 | 6652 | 1330 | | |
| Error | 25 | 19243 | 770 | | |
| Total | 35 | 52614 | | | |

SED = 16.02

LSD = 33.0

(ii) ANOVA on carbohydrate levels 3 months after treatment (Figures 5.5-5.7)

Reducing sugars

| Source | df | SS | MS | F | Sign. |
|---|---|---|---|---|---|
| PBZ | 5 | 643.1 | 128.6 | 1.36 | NS |
| Plant part | 2 | 4442.9 | 2221.5 | 23.53 | *** |
| Error | 10 | 933.7 | 94.4 | | |
| Total | 17 | 6019.7 | | | |

SED (PBZ) = 17.28

SED (Plant part) = 5.92

LSD (Plant part) = 12.60

Non-reducing sugars

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 5 | 256 | 51 | 0.50 | NS |
| Error | 11 | 1124 | 102 | | |
| Total | 16 | 1380 | | | |

SED = 8.25

| | | | | | |
|---|---|---|---|---|---|
| Plant part | 2 | 232.1 | 116.1 | 1.42 | NS |
| Error | 14 | 1148.0 | 82.0 | | |
| Total | 16 | 1380.1 | | | |

SED = 5.23

Starch

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 5 | 2738 | 548 | 0.35 | NS |
| Error | 11 | 17006 | 1546 | | |
| Total | 16 | 19744 | | | |

SED = 32.10

| | | | | | |
|---|---|---|---|---|---|
| Plant part | 2 | 13161 | 6581 | 14.0 | *** |
| Error | 14 | 6583 | 470 | | |
| Total | 16 | 19744 | | | |

SED = 12.52

LSD = 26.79

TNSC's

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 5 | 5349 | 1070 | 0.99 | NS |
| Plant part | 2 | 16556 | 8278 | 7.65 | ** |
| Error | 10 | 10821 | 1082 | | |
| Total | 17 | 32725 | | | |

SED (PBZ) = 39.00

SED (Plant part) = 18.96

LSD (Plant part) = 40.76

**(2D)** Stem extension growth on 7 month old plants following treatment with PBZ at 3 months of age.

| PBZ rate | Plant height mm |
|---|---|
| 0 | $82.9^{b}$ |
| 5 | $79.6^{b}$ |
| 10 | $74.6^{b}$ |
| 20 | $72.1^{b}$ |
| 40 | $55.8^{ab}$ |
| 80 | $25.8^{a}$ |
| SED | 16.96 |
| LSD | 34.27 |

(b) ANOVA of data on stem extension growth.

| Source | df | SS | MS | F | Sign. |
|---|---|---|---|---|---|
| Squares | 1 | 5168 | 5168 | | |
| Rows in SQ1 | 5 | 6881 | 1376 | | |
| Rows in SQ2 | 5 | 4267 | 853 | | |
| Columns in SQ1 | 5 | 4081 | 816 | | |
| Columns in SQ2 | 5 | 8075 | 1615 | | |
| PBZ | 5 | 27524 | 5505 | 3.18 | * |
| Error | 45 | 77703 | 1726.7 | | |
| Total | 71 | 133699 | | | |

**(2E)** (a) Monthly stem extension growth (mm) of 4 months old plants following treatment with PBZ. Final growth data taken when plants were 8 months old.

| mg PBZ per | Time from treatment with PBZ (months) | | |
|---|---|---|---|
| plant | 1 | 2 | 4 |
| 0 | 40.7 | 59.3 | 67.1 |
| 0.5 | 25.0 | 58.3 | 77.5 |
| 1 | 45.0 | 70.0 | 86.0 |
| 2 | 31.0 | 54.0 | 71.0 |
| 5 | 30.7 | 52.1 | 65.7 |
| 10 | 20.8 | 38.3 | 64.1 |
| 20 | 30.0 | 45.3 | 69.3 |
| 40 | 40.0 | 52.5 | 55.0 |
| 80 | 27.1 | 35.7 | 40.7 |
| | | | |
| SED | 14.05 | 17.34 | 18.00 |
| SIGN. | NS | NS | NS |

(b) Node length and leaf area of plants at 8 months of age.

| PBZ rate mg/plant | node length mm | leaf area sq. cm | ext. growth mm |
|---|---|---|---|
| 0 | 18.05 | 206.5 | 67.1 |
| 0.5 | 16.39 | 230.0 | 77.5 |
| 1 | 23.13 | 250.6 | 86.0 |
| 2 | 16.68 | 247.1 | 71.0 |
| 5 | 14.17 | 192.8 | 65.7 |
| 10 | 14.86 | 205.6 | 64.1 |
| 20 | 14.92 | 237.0 | 69.2 |
| 40 | 13.27 | 263.9 | 55.0 |
| 80 | 11.32 | 250.7 | 40.7 |
| SED | 3.37 | 52.46 | 18.0 |

(c) ANOVA of data on growth of 4 month old seedlings following treatment with PBZ. All data taken 4 months after treatment. (Figs. 5.3-5.4)

(i) node length

| Source | df | SS | MS | F | Sign. |
|---|---|---|---|---|---|
| PBZ | 8 | 504.6 | 63.1 | 1.85 | NS |
| Error | 44 | 1497.4 | 34 | | |
| Total | 52 | 2002 | | | |

(ii) leaf area

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 8 | 28528 | 3566 | 0.43 | NS |
| Error | 44 | 363281 | 8256 | | |
| Total | 52 | 391809 | | | |

(iii) extension growth

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 8 | 7965 | 996 | 1.02 | NS |
| Error | 44 | 42755 | | | |
| Total | 52 | 50720 | | | |

**(2F)** ANOVA of data on the effects of PBZ on mature trees -(Tables 6.1-6.2 and Figs. 6.1-6.3)

(a) 1990

(i) Flush length

| Source | df | SS | MS | F | Sign. |
|---|---|---|---|---|---|
| PBZ | 3 | 2019.5 | 673.2 | 10.74 | *** |
| Blocks | 3 | 1208.3 | 402.8 | 6.42 | |
| Interaction | 9 | 797.7 | 88.6 | | |
| Error | 112 | 7025.6 | 62.7 | | |
| Total | 127 | 11051 | | | |

SED = 1.98

LSD = 3.88

(ii) Trunk girth

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 3 | 50.3 | 16.8 | 0.99 | NS |
| Blocks | 3 | 16.1 | 5.4 | | |
| Interaction | 9 | 261.3 | 29 | | |
| Error | 16 | 272.5 | 17 | | |
| Total | 31 | 600.2 | | | |

SED = 2.06

(iii) Canopy width

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 3 | 0.159 | 0.053 | 0.14 | NS |
| Blocks | 3 | 0.258 | 0.086 | | |
| Interaction | 9 | 2.533 | 0.281 | | |
| Error | 16 | 6.172 | 0.386 | | |
| Total | 31 | 9.122 | | | |

SED = 0.32

(iv) Nut yield

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 3 | 120269 | 40090 | 1.76 | NS |
| Blocks | 3 | 268211 | 89404 | | |
| Interaction | 9 | 891596 | 99066 | | |
| Error | 16 | 363469 | 22717 | | |
| Total | 31 | 1643545 | | | |

SED = 75.36

(v) % Recovery

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 3 | 203.0 | 67.7 | 1.62 | NS |
| Error | 26 | 1082.9 | 41.6 | | |
| Total | 29 | 1285.9 | | | |

SED = 3.22

(vi) % Grade 1

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 3 | 1216 | 405 | 0.99 | NS |
| Error | 26 | 10671 | 410 | | |
| Total | 29 | 11887 | | | |

SED = 10.10

(b) 1991

(i) Flush length

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 3 | 762.7 | 254.2 | 24.9 | *** |
| Blocks | 3 | 15.9 | 5.3 | | |
| Interaction | 9 | 46 | 5.1 | | |
| Error | 144 | 1464.6 | 10.2 | | |
| Total | 159 | 2289.3 | | | |

SED = 0.71

LSD = 1.39

(ii) Nut yield

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 3 | 29763 | 9921 | 4.23 | * |
| Blocks | 3 | 7313 | 2437 | | |
| Interaction | 9 | 43013 | 4779 | | |
| Error | 16 | 37500 | 2344 | | |
| Total | 31 | 117588 | | | |

SED = 24.21

LSD = 51.32

(iii) % Recovery

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 3 | 2.02 | 0.67 | 0.07 | NS |
| Blocks | 3 | 42.52 | 14.17 | | |
| Interaction | 9 | 307.9 | 34.21 | | |
| Error | 16 | 153.74 | 9.61 | | |
| Total | 31 | 506.17 | | | |

SED = 1.55

(iv) % Grade 1

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 3 | 1293.3 | 431.1 | 5.12 | * |
| Blocks | 3 | 225.9 | | | |
| Interaction | 9 | 2383.4 | | | |
| Error | 16 | 1346.6 | 84.2 | | |
| Total | 31 | 5249.2 | | | |

SED = 4.59

LSD = 9.73

(v) Carbohydrate levels (Table 6.2)

Reducing sugars

| Source | df | SS | MS | F | Sign. |
|---|---|---|---|---|---|
| PBZ | 3 | 1857.0 | 619.0 | 1795.8 | *** |
| Replicates | 1 | 0.186 | 0.186 | | |
| Error | 3 | 1.089 | 0.363 | | |
| Total | 7 | 1858.3 | | | |

SED = 0.60

LSD = 1.91

Non-reducing sugars

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 3 | 321.6 | 107.2 | 4.89 | NS |
| Replicates | 1 | 31.6 | 31.6 | | |
| Error | 3 | 65.8 | 21.9 | | |
| Total | 7 | 419.0 | | | |

SED = 4.68

Starch

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 3 | 344.774 | 114.925 | 476.86 | *** |
| Replicates | 1 | 2.101 | 2.101 | | |
| Error | 3 | 0.724 | 0.241 | | |
| Total | 7 | 347.599 | | | |

SED = 0.49

LSD = 1.56

**(2G)** ANOVA of data on the effects of PBZ on root growth

(a) Root weight - Table 7.1

(i) 4 months old plants

| Source | df | SS | MS | F | Sign. |
|---|---|---|---|---|---|
| PBZ | 4 | 1.637 | 0.404 | 1.41 | NS |
| Blocks | 2 | 0.494 | 0.247 | | |
| Error | 8 | 2.317 | 0.290 | | |
| Total | 14 | 4.448 | | | |

SED = 0.44

(ii) 7 months old plants

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 5 | 31.739 | 6.348 | 8.55 | ** |
| Error | 58 | 43.056 | 0.742 | | |
| Total | 63 | 74.795 | | | |

SED = 0.36

LSD = 0.72

(b) Number of cluster roots - Table 7.1

Data transformed to square roots.

(i) 4 months old

| Source | df | SS | MS | F | Sign. |
|---|---|---|---|---|---|
| Blocks | 3 | 15.50 | 5.17 | | |
| PBZ | 4 | 47.82 | 11.95 | 5.09 | * |
| Error | 12 | 28.23 | 2.35 | | |
| Total | 19 | 91.54 | | | |

SED = 1.08

LSD = 2.35

(ii) 7 months old plants

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 5 | 48.02 | 9.6 | 5.72 | *** |
| Error | 59 | 99.05 | 1.68 | | |
| Total | 64 | 147.07 | | | |

SED = 0.55

LSD = 1.10

(c) Carbohydrates in 4 months old plants -(Table 7.2)

Reducing sugars

| Source | df | SS | MS | F | Sign. |
|---|---|---|---|---|---|
| PBZ | 4 | 785.276 | 196.319 | 934 | *** |
| Replicates | 1 | 0.044 | 0.044 | | |
| Error | 4 | 0.838 | 0.210 | | |
| Total | 9 | 786.158 | | | |

SED = 0.46

LSD = 1.27

Non-reducing sugars

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 4 | 288.1 | 72.0 | 2.69 | NS |
| Replicates | 1 | 4.9 | 4.9 | | |
| Error | 4 | 107.4 | 26.8 | | |
| Total | 9 | 400.4 | | | |

SED = 5.18

Starch

| | | | | | |
|---|---|---|---|---|---|
| PBZ | 4 | 420 | 105.0 | 10.4 | * |
| Replicates | 1 | 7 | 7.0 | | |
| Error | 4 | 40.5 | 10.1 | | |
| Total | 9 | 467.5 | | | |

SED = 3.18

LSD = 8.83